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FACTORS AFFECTING MATERNAL RH IMMUNISATION

BY

H. R. NEVANLINNA

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BY

H. R. NEVANLINNA

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PREFACE

In its original form this investigation envisaged a comparison of the frequency of Rh immunisation in mothers who had and who had not received blood transfusions. It was expected that a comparison of this kind would lead to conclusions concerning the strength of the immunisation stimuli resulting from blood transfusion on the one hand and pregnancy on the other. It soon became apparent, however, that this type of comparison made it imperative to take into account a great number of factors which influence immunisation frequency and which, therefore, play a decisive rôle in the ultimate results. Another conclusion reached during the initial stages of this work concerned the difference between immunisation frequency by transfusion and by pregnancy. A mere explanation on the ground of the differences in the strength of immunisation stimuli did not suffice: the difference had to be accounted for on the basis of characteristics peculiar to the mechanism governing pregnancy immunisation.

For these reasons the investigation now appears in a more theoretical form and covers various factors which affect maternal Rh immunisation. While I have attempted to deal with the question of the difference between the mechanisms of transfusion immunisation and pregnancy immunisation by taking into account the literature on the subject as well as by utilizing material collected by myself, I have also permitted myself to digress and speculate on some theoretical aspects of the problem.

It is with deep regret that I express my indebtedness to one who is no longer with us, Prof. M a u n o R a u r a m o, M. D., late Director of the Women's Clinic, Helsinki, who initially provided me with the opportunity of investigating questions relating to the Rh factor in Finland and who, up to the time of his death, supported my work wholeheartedly. I wish to thank also Prof. K. O. R e n k o n e n, M.D., Director of the Department of Serology and Bacteriology, University of Helsinki, for the active interest with which he has promoted my work throughout

the years. When I shifted the centre of my investigation to the State Serum Institute, Prof. Eero Uroma, M.D., the Director, made possible the expansion of the scope of my laboratory activities; this circumstance, which decisively contributed to the progress of my work, enabled me to gather a considerable quantity of material. Since cases of erytroblastosis are, for the most part, treated at the Children's Clinic, I have also enjoyed the opportunity of studying cases at that hospital, thanks to the kind interest of the hospital's Director, Prof. Arvo Ylppö, M.D., Prof. C.-E. Räihä, M.D., and Dr. Niilo Hallman. Likewise, I am indebted to Prof. Aarno Turunen, M.D., Director of the Women's Clinic, the centre for the care of Rh immunised mothers.

Part of the material presented has been gathered at the Women's Clinic, the Finnish Red Cross Hospital (Prof. Aarno Snellman, M.D., Director), and the Institute of Midwifery (Prof. A. Apajalahti, M.D., Director). To all these I express my thanks for the favourable attitude which they displayed toward my researches.

My work has also benefited from the interest and advice of many colleagues at the State Serum Institute and at the University's Department of Serology and Bacteriology. It is with special gratitude that I mention my colleague, Dr. Erik E. Anttinen, with whom I have worked side by side for many years and who has contributed to a great extent to the present investigation. My conversations with him and with Dr. Kari Penttinen and Dr. Odd Wager have provided me with stimulation and the necessary spirit of criticism.

My assistants at the Blood Grouping Laboratory and at the Finnish Red Cross Blood Service have been my faithful companions throughout my researches. Mrs. Kaisu Lehtonen, R.N., has energetically carried out the perusal of the card indexes as well as many other technical tasks, the most important of which was the securing of samples from the members of Rh negative mothers' families. The personnel of the various wards, operating rooms and laboratories of the Children's Clinic and the personnel of the Women's Clinic's maternity wards are all due thanks for the successful care of the patients. Unfortunately space prevents me from mentioning the great number of

physicians, midwives and public nurses whose information and consignments of samples have made this study possible.

Throughout the years I have also received help from abroad, especially from the other Scandinavian countries. I have had occasion to benefit from the advice of Dr. Birger Broman, Stockholm, who has also placed test sera at my disposal and permitted me to witness the operation of his laboratory. Dr. Otto Hartmann, Oslo, and Dr. Erik Freiesleben, Copenhagen, have followed my work with interest, placed their materials at my disposal, and provided me with test sera.

Finally, I wish to thank Mrs. Hilkka Kontiopää, Mag. Phil., and my wife, Saara Nevanlinna, Mag. Phil., for translating the manuscript into English, Mr. L. Keyworth, M.A., Cantab., and Mr. Robert Austerlitz, M.A., for checking the translation.

I have received considerable financial aid from Suomen Kulttuurirahasto (The Finnish Culture Foundation).

Kilo, October, 1952.

H. R. Nevanlinna.

CONTENTS

PREFACE III

INTRODUCTION 3

OBJECT OF THE PRESENT INVESTIGATION 7

Chapter I. IMMUNISATION INDUCED BY REPEATED PREGNANCIES 9

1. Pregnancy as an immunisation stimulus 9
2. Frequency and probability of pregnancy immunisation 12
3. Frequency of immunisation and factors affecting it .. 14
   a) Selection 15
   b) Probability of immunisation and fertility of immunised patients 16
   c) Arrangement and methods of investigation 18
   d) Differences in the relative sizes of the different parous groups in the material 19
   e) D and d gene frequencies in the material (population) 22
4. The relative number of the homozygous husbands of immunised patients 24

Chapter II. IMMUNISATION THROUGH BLOOD TRANSFUSION 27

1. Frequency of immunisation 27
2. Follow-up examination of transfused female patients .. 29
   a) Purpose of the work 29
   b) Material 29
   c) Evaluation of the tests, and nomenclature 30
   d) Results 31
   e) Technique 42
   f) Conclusions 45
   g) Summary of the results of the follow-up examination 46
3. Effect of blood transfusions on HDN frequency 47
4. Effect of a blood transfusion given to the mother on the prognosis of the child 48
5. Does the foetus act as an immunisation stimulus in transfused mothers? 50
6. Intramuscular blood injections 52

Chapter III. ON FACTORS PROMOTING AND INHIBITING PREGNANCY IMMUNISATION 55

1. Survey of literature 55
2. Summary of Chapters I and II 57

3. Maternal constitutional factors 58
4. Obstetrical factors 59
5. Foetal factors 60
The foetal factor inhibiting sensibilisation
a) Effect of the sensibilisation-inhibiting factor on the frequency of immunisation 62
b) Effect of the sensibilisation-inhibiting factor on the probability of immunisation 64
c) A and B blood group factors as examples of a foetal factor inhibiting sensibilisation 67
6. Discussion 70

SUMMARY 72

BIBLIOGRAPHY 77

INTRODUCTION

The clinical manifestations of erythroblastosis, foetal hydrops, icterus gravis and anaemia gravis were first described as separate diseases of the foetus and the newborn late in the last or early in this century [2,1,7]. However, these different forms of the disease were not connected with one another until the 1930's [18,14,15] although attention had already been devoted much earlier to the familial occurrence of the disease [2,1].

Various assumptions had early been advanced regarding the etiology of the disease. Among them may be mentioned Ylppö's [81] work on icterus gravis, maintaining that the etiology of the disease can be explained from the immaturity of the liver of the newborn. Gradually, increasing attention was devoted to haemolysis and compensatory erythropoiesis, the existence of which was supported by several symptoms found in the different forms of the disease. Various theories were advanced on the causes and mechanism of haemolysis, but Darrow [12] was the first to consider that the different symptoms of the disease were explicable from the antigen-antibody reaction in the infant. He thought it possible that the mother had become immunised against some foetal antigen, the antibodies that developed subsequently passing through the placenta into the infant causing haemolysis. He suggested that the haemoglobin of the foetal period might be such an antigen; it was by then known to possess qualities different from the haemoglobin of the adult.

As early as 1905 Dienst [23] concluded that the A and B blood group factors of the foetus may induce a rise in maternal anti-A and anti-B titres if the corresponding factor is absent in the mother. Jonsson [17] found a rise in anti-A and anti-B haemolysins in the mother in such heterospecific pregnancies.

In 1940 Landsteiner and Wiener [33] published their results of investigations effected with immune rabbit serum, with red cells of the Rhesus monkey employed as the antigen in the immunisation. Using an absorbed serum they were able to find in the red cells of most of their subjects an agglutinogen which they

termed blood group factor Rh. Soon afterwards Wiener and Peters [67] found Rh antibodies in three patients with serious transfusion reactions. As the patients had already either received blood transfusions or been pregnant earlier, they concluded that antibodies may be produced in two different ways: the red cells enter the patient's blood circulation either through blood transfusion or from the foetus via the placenta.

Levine and his co-workers had attained the same results at the same time as Wiener, though independently. Apart from the fact that he found Rh agglutinins after transfusion complications [34], Levine was the first to explain the etiology of erythroblastosis from Rh immunisation of the mother [35,36]. A little later [37], having collected 153 cases in which the mother had given birth to an erythroblastotic infant, he found good grounds for his theory that the foetal Rh antigen inherited from the father immunises the mother, and the Rh antibodies, on entering the foetus, cause erythroblastosis.

It must be considered a remarkable achievement on the part of Wiener and Levine to explain in such a short space of time the etiology of many transfusion complications and of erythroblastosis by means of theories which still hold good. Below a few of the most important steps which have made a decisive contribution to our knowledge of the blood groups and their influence on the haemolytic disease of the newborn will be pointed out.

The original observations by Levine and other investigators supported his theory of the etiology of erythroblastosis in the first place statistically: almost all the erythroblastotic infants were found to be Rh positive, the majority of the mothers Rh negative. On the other hand, serologically demonstrable anti-Rh agglutinins could only be shown in certain cases, fewer in number the longer the time that had elapsed from delivery. Although maternal immunisation could be considered probable in all cases of erythroblastosis [38,6], the final solution came when Race [53] and Wiener [22], independently, found that Rh antibodies occur in two different forms. The practical importance of the blocking or »incomplete» antibodies increased in the following years as their serological determination became more reliable and sensitive [19,20,10,73,48]. Hence, the validity of Levine's theory could

be established both statistically and casuistically: Rh antibodies could be found in all cases of immunisation; insofar as Rh antibodies could not be demonstrated in the mother's serum there could be no question of Rh immunisation and the haemolytic disease of the newborn caused by it.

The very sensitive technique of antibody determination, improved in this way, caused little change in the conception of the frequency of the maternal Rh immunisation through repeated pregnancies. Diamond [21] was the first to show conclusively, by means of the technique he had developed, that transfusions of Rh positive blood very often resulted in the immunisation of Rh negative patients: in half the transfused patients examined by him Rh antibodies could be demonstrated years later. Aside from the great practical importance of this observation in transfusion treatment, it showed that the rarity of Rh immunisation could not be due to a general incapacity to develop Rh antibodies.

The existence of an antigen-antibody reaction in the infant received additional confirmation when Coombs [10,11], using the technique developed by himself, showed that antibodies derived from the mother were attached to the infant's red cells. Indicative of the same thing were the numerous observations of the favourable influence on the course of the disease of transfusions of Rh negative blood and particularly of exchange transfusion. Apart from numerous clinical observations, Mollison [42] carried out a very remarkable experimental investigation: he showed that the Rh positive red cells had a distinctly shortened survival time in an erythroblastotic child, whereas the disease in no way affected the life of transfused Rh negative red cells, which remained normal.

It had already become evident in the initial phases of Rh research that qualitatively different antibodies developed as a result of immunisation; their origination must have been due to the influence of different Rh antigens. Fisher and Race [54,55] worked out their well-known »eight-gene« theory, originally based on the antithesis of two known antigens. According to them, an Rh chromosome has three genes close to one another but sufficiently apart for crossing-over to occur, though extremely seldom. Wiener [71,70,69,74] again considers that a single locus with multiple

allelomorphs is in question. From the practical point of view, the significance of these differing views is confined to two different nomenclatures being used either together or separately. In the present work, either both nomenclatures or only the English have been used. The essential features of the two nomenclatures are given on p. 31.

Although, in addition to Rh antigens, many other blood group antigens may induce isoimmunisation either as a result of repeated pregnancies or of blood transfusions [57], D antigen [44] is definitely the most common in this respect. It may be said that familial erythroblastosis in the vast majority of cases is due to the maternal isoimmunisation caused either by D antigen alone or by D together with other antigens. This, in the present author's opinion, is what justified limiting the present investigation to the maternal immunisation caused by the D factor alone.

At the moment we know the essential conditions for the haemolytic disease of the newborn (HDN): Rh antigen and its specific antibody must be present simultaneously in the foetal organism. In addition, most of the symptoms of the disease are readily explicable from the intravascular haemolysis caused in the foetus and the newborn infant by the antigen-antibody reaction, and from the consequences of this haemolysis. The fact that some symptoms have no distinct correlation with the haemolytic anaemias of later age need not mean that these symptoms arise from something else than haemolysis: the haemolytic disease of the newborn of course develops in the special conditions of the foetal period, conditions which are suddenly changed at the moment of birth.

Although more is known now of the etiology and pathogenesis of HDN than of the majority of the other diseases of the newborn and even of adults, the prerequisites for placental immunisation are not known. This became evident after it was found that most Rh negative persons, as a result of blood transfusions, are well capable of forming antibodies. The factors that are responsible for certain of the special features of pregnancy immunisation and expressly for its rare occurrence are doubtless still for the greatest part veiled in obscurity.

OBJECT OF THE PRESENT INVESTIGATION

Many excellent monographs have been written about the significance of Rh groups in blood group research and clinical medicine [68,6,51,56,49,43], giving complete bibliographies of all the most important investigations into the subject. The present work, for this reason, is not intended to be a monograph; it confines itself to a theoretical discussion of the nature and mode of action of the factors affecting pregnancy immunisation and its mechanism. The purpose of the paper is

— to study in greater detail certain features of the immunisation mechanism important from the point of view of pure pregnancy immunisation,

— to describe how a blood transfusion given to a female patient affects subsequent pregnancy immunisation, and

— on the basis of the above, to endeavour an explanation of the rare occurrence of pregnancy immunisation compared with transfusion immunisation.

Some basic conceptions important from the point of view of immunisation have been found to require clarification, e.g., in order to make various materials comparable. For this reason the nature of the factors affecting the frequency of pregnancy immunisation and the extent of their effect have been dealt with in detail. At the same time attention has been paid to the changes caused by these factors in the relative numbers of homozygous and heterozygous husbands among the immunised patients.

CHAPTER I

IMMUNISATION INDUCED BY REPEATED PREGNANCIES

1. Pregnancy as an Immunisation Stimulus

According to Levine's theory Rh immunisation is produced by the foetal red cells entering the maternal circulation through the placenta. There is nothing in later investigations to contradict this hypothesis; we do not know, however, whether we have to deal with an antigen normally entering the maternal circulation in all pregnancies or whether the process is confined to a few pathological cases.

In any case it is certain that Rh immunisation is observed during the first pregnancy only very rarely: at least two pregnancies with Rh positive foetuses are necessary for immunisation to develop. The primary importance of the first pregnancy in the immunisation process has no doubt mostly been overlooked. The Rh antigen must escape from the foetus during the first pregnancy (or delivery), resulting in a change in the mother's mode of reaction. The immunisation will thus manifest itself due to the influence of the next Rh positive foetus. This change in the mode of reaction, which is not serologically detectable as a rule, will be called *sensibilisation* in the present investigation. Sensibilisation must undoubtedly be regarded as the basic condition for pregnancy immunisation proper.

Repeated pregnancies seldom, according to Mollison's [56] calculation in about 10 per cent of the cases only, cause the immunisation of an Rh negative mother, even when she has borne two or even more Rh positive children. At first sight it may seem as if pregnancy as an immunisation stimulus is not in most cases strong enough to cause immunisation.

Immunisation, however, is very seldom manifested gradually; the antibody titre rising gradually owing to the action of several

pregnancies finally results in manifest haemolytic disease in the newborn. By contrast the disease is generally severe even in the child first causing manifest immunisation and the antibody titre in the mother is remarkable. Subclinical cases will not, as a rule, be repeated in one and the same family; they are mostly due probably to the late development of antibodies during pregnancy.

The following table shows the prognosis of the diseased children in 246 cases of maternal Rh immunisation observed at the Blood Grouping Laboratory of the State Serum Institute in 1948—1951 The material is classified according to the consecutive order of the diseased children.*

TABLE 1

	Consecutive order of the sick child						
	I	II	III	IV	V	Total	
Recovered	81	37	17	11	6	*152*	(30.0 %)
Exchange transfusion	16	18	9	8	5	*56*	
Blood transfusion(s) .	20	5	—	1	—	*26*	
No transfusion	45	14	8	2	1	*70*	
Died	165	96	50	22	22	*355*	(70.0 %)
Exchange transfusion	1	2	1	—	—	*4*	
Blood transfusion(s) .	10	—	—	1	—	*11*	
No transfusion	93	46	29	9	4	*181*	
Stillborn	61	48	20	12	18	*159*	
Total	*246*	*133*	*67*	*33*	*28*	*507*	

Pattern of the disease in 246 cases of Rh immunisation. The cases where the mother had a history of blood transfusion are excluded.

Although the mortality increases in this material after each diseased child, the prognosis even of the first diseased child is rather bad. But the material does not, it is true, include some

* Only cases where at least one of the children has apparently been affected with HDN are included in the material. Some of the cases have been clinically examined and treated by the present author; the diagnosis is partially based on symptoms observed clinically or on autopsy and partially on the medical history provided by the patient herself. All the cases where the mother had had a transfusion before the first sick child are excluded, as well as those in which the medical history was insufficient. The Rh negative children have been excluded from the consecutive order of the diseased children.

few mild and subclinical cases. Since, however, over half of the cases were immunised during the second pregnancy (see Table 3), pregnancy must be regarded as a *very good immunisation stimulus* in cases where immunisation has been manifested.

The following table illustrates the prognosis of the first diseased child in cases when it is preceded by only one healthy child compared with cases in which two or more healthy children preceded the first diseased one. The material is the same as that presented in Table 1, only the cases with an abortion prior to the diseased child being excluded.

TABLE 2

	Number of cases where the 1st sick child is preceded by only one healthy child	Number of cases where the 1st sick child is preceded by two or more healthy children
Recovered	*40* (30 %)	*25* (32 %)
Exchange transfusion	8	4
Blood transfusion(s)	12	12
No transfusion	20	9
Died	*94* (70 %)	*53* (68 %)
Exchange transfusion	—	—
Blood transfusion(s)	6	4
No transfusion	59	27
Stillborn	29	22
Total	*134*	*78*

Prognosis of the first sick child in 134 cases of Rh immunisation where the first sick child is preceded by only one healthy child compared with 78 cases where the first sick child is preceded by two or more healthy children. Cases where the mother had a history of blood transfusion or abortion before the onset of the disease are excluded.

The prognosis of the first diseased child being much the same in each group of the material, it is unlikely that part of the Rh positive foetuses in the latter group acted as sensibilising stimuli at all. This would account for the late manifestation on the immunisation in the latter group, even if immunisation had developed in each group as a result of the minimum number of stimuli, i.e., only two.

2. Frequency and Probability of Pregnancy Immunisation

The frequency of immunisation implies, as is known, the ratio between immunised patients and the total number of patients (or the Rh negative) examined. This frequency can be computed for the whole material or for each parous group separately, insofar as the number of deliveries of each patient examined is known. The immunised in a certain parous group can be further divided into two groups: those immunised for the first time during the pregnancy under investigation and those immunised during the preceding or during a still earlier pregnancy.

In the following *the probability of immunisation* refers to the probability with which a patient is first immunised during a certain pregnancy. In calculating the probability of immunisation cases immunised during earlier pregnancies must obviously be excluded. Disregarding the few cases immunised during the first pregnancy, the frequency and probability of immunisation are equally high in secundiparae. Later the frequency will always be higher than the probability; when computing the frequency of immunisation in III-parae, besides those first immunised during the third pregnancy also those immunised during the second pregnancy, if they are again pregnant at the moment of investigation, must be included, etc.

Although the factors affecting the frequency of immunisation will not actually be treated until later, it may be stated in this connection that the frequency of immunisation depends on both the probability of immunisation and the probability of an already immunised patient giving birth to another child. The latter probability may be termed the *fertility of immunised patients.*

Let us denote the fertility of the whole material (or population) by 1. If the fertility of the immunised patients is the same as in the whole material, i.e. 1, the frequency of immunisation is the same as the probability of immunisation in the second pregnancy, double in the third pregnancy, three times as high in the fourth and so on. Naturally the frequency of immunisation can be estimated even when the fertility of the immunised differs from the fertility of the whole material, provided

that the probability of immunisation in each parous group is known.

The absolute probability of immunisation value can only be estimated if the total number of patients examined belonging to the parous group in question is known. A largely correct picture of the variation of this probability in different parous groups can be obtained even without that, provided the immunisation material is collected evenly from a population with known relative parous group sizes. From the immunisation material a calculation is made of the percentage in which immunisation has first manifested itself during the first, second, third, etc. pregnancy respectively. The percentual figures are compared with the relative sizes of the corresponding parous groups. This gives a value for the relative probability of immunisation. Reduced to a whole number the value can be termed the *index of immunisation probability*.

The variation of the relative probability of immunisation in the different parous groups is calculated in the following table from the material presented in Table 1. Abortions too have been taken as pregnancies in the material, but cases with a history of blood transfusion have been excluded. The relative probability of immunisation and its index is calculated as described above. The relative parous group sizes are those in Finland in 1949. Both live and stillbirths in wedlock and out of wedlock are included.

TABLE 3

Parity	Number of cases	Per cent	Parous groups in Finland 1949	Index of probability
I	2	0.8	32.2	2
II	131	53.3	26.6	201
III	68	27.6	16.8	164
IV	28	11.4	9.2	124
V	10	4.1	5.5	75
VI	4	1.6	3.4	47
VII	1	0.4	2.3	17
VIII≷	2	0.8	4.0	
Total	*246*	*100.0*	*100.0*	—

Consecutive order of the pregnancy in which immunisation was first manifested, and the index of probability in 246 cases of pregnancy immunisation. The relative sizes of the different parous groups are calculated from live and stillbirths in Finland 1949, in wedlock and out of wedlock.

According to this material, the probability of immunisation declines quite radically from the second pregnancy onwards. The decline is more radical still if the fact is taken into consideration that a number of husbands are heterozygotes (with immunisation consequently delayed) and that a number of the cases have undoubtedly already been immunised in the previous pregnancy, although, owing to the mild symptoms, this has passed unnoticed.

It is difficult to compare these results with materials previously published. Van Loghem [66] has calculated from his material the pregnancy in which immunisation was first manifested, but included in his material cases where the mother was transfused before the birth of the first affected child. However, if his figures are compared with the relative parous group sizes in Holland, the probability of immunisation declines, although more slowly and irregularly than in the present material. Potter [51] found that the immunisation probability rose in her hospital material up to the fifth pregnancy and then declined. Even this observation cannot be directly compared with the present material because the principles of the calculations are likely to have been different.

3. Frequency of Immunisation and Factors Affecting it

During the last ten years many estimations have been made of the frequency of HDN. Most of these, however, deal with clinical erythroblastosis only. Further, materials have often been collected from hospitals. For this reason they are in many respects selected and cannot be compared with materials collected from the whole population or from certain sections of it. According to Schwarz and Levine's [59] estimation the disease occurred once in 200 deliveries, in Potter's [51] extensive material the frequency was 1: 242 and in Pickles's [49] 1: 378 in 1939—47. Later investigations give a more accurate picture of the frequency of immunisation, based as they are on extensive routine investigations into relatively unselected materials of pregnant women. In Pickles's [49] material the frequency of immunisation was 1: 155 and in Hartmann's [87] 1: 145.

Many authors have pointed out several factors which make it difficult to estimate the real frequency of immunisation or HDN. From a clinical point of view, however, the results arrived at may be considered sufficiently accurate for an estimation of the effect of HDN on infant mortality, of the frequency of stillbirths or other clinical problems. If the matter is considered from a purely theoretical point of view, so many known factors (variations in the number of primiparae, selection, the difference between the frequency of immunisation and that of HDN, the frequency of transfusion immunisation etc.) obviously affect the results obtained from different materials in so many different ways that they cannot be compared, at least not directly. Two questions, above all, are of theoretical importance:

1. How many Rh negative women are able to form Rh antibodies with repeated pregnancies as the only immunisation stimulus?
2. Is the »corrected frequency of immunisation» thus obtained the same in different materials or are there variations so distinctive that they might induce the investigator to believe that other factors independent of the Rh factor affect pregnancy immunisation?

The following is not intended to provide an answer to these questions, which indeed is not possible within the limits of this investigation. It is the author's intention only to discuss in detail the different factors which affect the frequency of immunisation in a certain material and to estimate the effect of these factors as accurately as possible.

a) *Selection*

When estimating the frequency of pregnancy immunisation all those cases should naturally be excluded in which the mother has received transfusions or intramuscular injections of blood. In many materials the number immunised by transfusions may amount to several dozen per cent of all the immunisation cases (see p. 47).

Because Rh immunisation in most cases results in a clinical manifestation, erythroblastosis, it can be presumed that some immunised patients for this reason seek treatment elsewhere,

though they actually belong to the material under investigation, while others are included in the material for the same reason; it is difficult to define the reasons in each case which cause a patient to submit herself for examination at a certain hospital or institute.

A very important point is whether cases in which immunisation has been observed during preceding pregnancies should be included in the material or not. Rh investigation has become so common now that it is difficult to collect a material in which no Rh investigations have been made earlier, e.g. when the medical history was suspect. If cases immunised during preceding pregnancies are included we cannot, naturally, disregard those in which the diagnosis has earlier been confirmed through Rh investigation. Such a policy would lead to an artificial decrease in the number of immunised multiparae.

b) *Probability of immunisation and fertility of immunised patients.*

Estimates of the frequency of immunisation have usually assumed that the fertility of immunised patients is equal to that of the whole material, i.e. that the probability of an immunised patient giving birth to one more child is the same as that of others in the same parous group, which gives a fertility index of 1. The situation may not, however, be quite so simple. As there is no material available that would illustrate the problem, the following estimation will be rather speculative.

It seems likely that giving birth to one or perhaps even two diseased children would stimulate the family to make another effort to have a healthy child, unless the family is informed of the familial character of the disease. The index of fertility of the immunised patients would thus be 1 or more to begin with. Later one would expect the fertility to decline radically; a mother who has given birth to three or more sick children will realise the hopelessness of the situation at this stage even without any special investigation.

As mentioned above, the frequency and probability of immunisation are two different kinds of concept. The frequency has a definite dependence on the probability of immunisation as well as on the fertility of the immunised patients. If both these are known for the parous group in question and also for the preced-

ing one, the frequency of immunisation can be calculated simply. This may be illustrated by the following equation:

$$I_n = F_{n-1} \cdot P_{n-1} + P_n$$

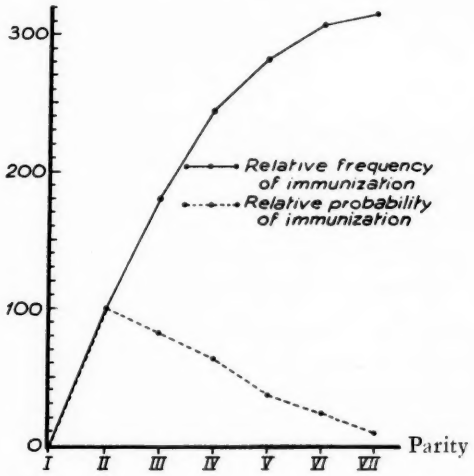
where I = frequency of immunisation
F = fertility of immunised patients
P = probability of immunisation
n = consecutive order of pregnancy

If the fertility of immunised patients after two deliveries is e.g. 1.25, the probability of immunisation of Rh negative patients in the third pregnancy 0.04 and in the second pregnancy 0.05 (1:25 and 1:20) the frequency of immunisation with tertiparae will be

$$I_3 = 1.25 \cdot 0.05 + 0.04 = 0.1025$$

Because the fertility of immunised patients apparently depends greatly on how many diseased children the patient has already had, the calculation given above is hardly of any practical value. The following diagram illustrates how sharply the frequency of immunisation increases when the relative fertility remains un-

DIAGRAM I



Frequency and probability of immunisation when the relative fertility remains unchanged in the different parous groups. The probability of immunisation values are taken from Table 2.

changed in the different parous groups, although the probability of immunisation declines (F = 1). The probability of immunisation values are taken from Table 2 and are consequently only relative.

In Pickles's [49] material the frequency of HDN rose between the second and the fifth pregnancy from 1: 43 to 1: 20. If it is assumed that the probability of immunisation was the same as in the present author's material, the fertility of the immunised patients in Pickles's material was somewhat lower than the fertility of the normal population. Pickles's observation that the frequency of the disease declined from the sixth pregnancy onwards might result from a simultaneous radical decline in fertility while the probability of immunisation in these parous groups was approaching zero.

As a practical point it may be mentioned that as Rh investigation is extended to cover most pregnant women in many countries the fertility of immunised patients is declining, thus leading to a lower frequency of HDN.

c) *Arrangement and method of investigation*

The number of observed cases of immunisation depends to some extent on the time of examination of a pregnant patient. If an antibody determination is carried out in connection with the first visit to the maternity clinic and then about one month before term, a few cases of immunisation (though clinically mild) will be overlooked. In some cases pregnancy is interrupted by abortion even before the first investigation. The number of cases in which the antibodies appear after the first examination but in which no control has been carried out before term owing to neglect or premature delivery can be kept very low by careful organisation of the investigation.

Year by year the methods of antibody determination have been improved. If the Coombs indirect test and trypsinised control cells are used very similar results will be obtained from different materials. It has not, however, been possible to use such a sensitive technique in the older materials and therefore the figures for the frequency of immunisation have remained too low.

d) *Differences in the relative sizes of the different parous groups in the material*

The relative sizes of the different parous groups vary greatly from country to country. Even in one and the same country variation in this respect is recorded for different parts of the country and in different years, especially with the birth rate varying from year to year owing to war, crises etc. The relative sizes of the different parous groups in Finland in 1940—1950 are given in Table 4[8].

TABLE 4

	Parity							
Year	I	II	III	IV	V	VI	VII	VIII or more
1940	35.33	21.80	13.91	8.93	6.09	4.57	3.33	6.04
1941	32.80	24.55	15.06	9.23	6.32	4.25	2.89	4.90
1942	34.46	22.29	15.08	9.19	6.25	4.09	3.16	5.58
1943	32.57	23.21	15.45	9.62	6.29	4.39	3.02	5.44
1944	36.32	22.65	14.44	8.96	5.77	3.96	2.66	5.24
1945	38.61	23.98	14.05	8.44	5.19	3.40	2.23	4.10
1946	41.36	23.21	13.69	7.97	4.82	3.09	2.08	3.78
1947	38.29	26.05	14.04	8.11	4.80	3.15	2.02	3.54
1948	34.93	27.21	15.26	8.56	4.96	3.23	2.09	3.76
1949	32.27	26.60	16.78	9.20	5.50	3.43	2.32	3.90
1950	31.16	25.85	17.47	9.96	5.67	3.54	2.95	4.06

Relative sizes of the parous groups in Finland in 1940—1950.[1] Live and stillbirths in wedlock and out of wedlock.

Unfortunately it is not easy to estimate numerically the effect of the factors mentioned in a, b, and c above on the frequency of immunisation. But it is somewhat easier to calculate how the variation in the relative sizes of the different parous groups affects this frequency. It can at least be computed how many of the Rh negative patients in a certain material fulfil the minimum condition for immunisation, i.e. how many of them at the time of investigation have at least two Rh positive children. If we assume that the Rh negative percentage of the population is 16, with 48 % positive heterozygotes and 36 % homozygotes, we can calculate how many Rh negative mothers delivered in Finland in 1949 had at least two Rh positive children. The number

of Rh positive children by heterozygous fathers has been calculated from the formula $(m + n)^{1,2,3}\ldots$ in which m and n are equal. The sum of the combinations in each parous group is made = 100. The number of heterozygous fathers that have at least two D positive children is accordingly, e.g. with III-parae, 12.5 + 37.5 % = 50 %, with IV-parae 6.25 + 25 + 37.5 % = 68.75 % etc.

TABLE 5

Parity	Per cent	Father homozygote ‰	Father heterozygote ‰	Total ‰
I	32.2	—	—	—
II	26.6	95.8	31.9	127.7
III	16.8	60.5	40.3	100.8
IV	9.2	33.1	30.2	63.3
V	5.5	19.8	21.4	41.2
VI	3.4	12.2	14.7	29.9
VII	2.3	8.3	10.3	18.6
VIII≷	4.0	14.4	18.6	33.0
Total	*100.0*	*244.1*	*167.4*	*411.5*

The number of Rh negative mothers delivered in 1949 that have given birth to a minimum of two Rh positive children, calculated per 1 000 Rh negative mothers. Live and stillbirths in Finland in 1949, in wedlock and out of wedloock.

The result thus obtained shows that only about two fifths of the Rh negative in a material of this kind have given birth to at least two Rh positive children. How greatly different materials can vary even in one country in this respect becomes evident from a comparison between the numbers of Rh negative mothers with at least two Rh positive children in Helsinki and in northern Finland, for instance (Table 6).

If it is assumed, to illustrate the matter, that the figures above are directly proportional to the frequency of immunisation and that this frequency is 1: 200 in Helsinki, it would be about 1: 93 in the northern parts of the country.

TABLE 6

Helsinki 1950

Parity	Per cent	Father homozygote ‰	Father heterozygote ‰	Total ‰
I	53.4	—	—	—
II	25.3	91.0	30.2	121.2
III	10.2	36.7	24.5	61.2
IV	3.3	11.9	10.9	22.8
V	1.7	6.1	6.6	12.7
VI	0.7	2.5	3.0	5.5
VII	0.5	1.8	2.2	4.0
VIII≦	0.9	3.2	4.2	7.4
Total	*100.0*	*153.2*	*81.6*	*234.8*

Northern Parts of Finland 1950

Parity	Per cent	Father homozygote ‰	Father heterozygote ‰	Total ‰
I	21.9	—	—	—
II	22.5	81.0	27.0	108.0
III	18.0	64.8	43.2	108.0
IV	11.9	42.8	43.2	86.0
V	7.3	26.3	28.5	52.8
VI	5.8	20.9	24.8	45.7
VII	4.0	14.4	18.0	32.4
VIII≦	8.4	30.1	39.6	69.7
Total	*100.0*	*280.3*	*224.3*	*504.6*

Number of Rh negative mothers delivered in Helsinki in 1950 that have given birth to a minimum of two Rh positive children, calculated per 1 000 Rh negative mothers, compared with the corresponding figure obtained for deliveries in the northern parts of Finland (Oulun and Lapin lääni) in the same year. Live and stillbirths in wedlock and out of wedlock.

The figures of the relative sizes of parous groups obtained from different countries cannot always be compared with the figures above owing to different calculation principles. The following table shows the relative parous groups sizes in a few countries in 1948[16], as well as the number of Rh negative mothers who have given birth to a minimum of two Rh positive children.

TABLE 7

	United Kingdom per cent	Finland per cent	Netherlands per cent	Norway per cent
I	41.1	34.8	28.3	37.4
II	30.9	27.3	24.0	28.8
III	14.1	15.4	16.3	16.9
IV	6.1	8.7	11.3	8.2
V	3.1	5.0	7.1	4.1
VI	1.7	3.2	4.6	2.1
VII	1.0	2.0	3.1	1.1
VIII≷	1.5	3.6	5.3	1.4
	‰ 332.1	‰ 394.2	‰ 444.0	‰ 363.8

Relative sizes of the different parous groups in the United Kingdom, Finland, the Netherlands and Norway in 1948 and the number of Rh negative mothers that have given birth to a minimum of two Rh positive children. Live and stillbirths in wedlock and out of wedlock.

Considering that the 1948 birth rate in the United Kingdom was 18.1 pro mille and in Finland 27.2, and assuming that the figures given in Table 7 are still directly proportional to the frequency of immunisation, HDN is nearly twice as common a disease in Finland as in the United Kingdom.

Many materials may show remarkable differences in the relative sizes of the parous groups from the statistics given above of whole populations. When comparing two different materials it is therefore necessary to know the composition of the materials in this respect. E.g. Pickles's material had 60 per cent primiparae. Thus the number of Rh negative mothers with at least two Rh positive children was very much lower than in Hartmann's material, for instance, collected from all over the country.

e) *D and d gene frequencies in the material (population).*

Most of the Rh investigations performed have been on Indo-European populations, in which the Rh negative frequency does not vary much. It may, however, be interesting to examine the way in which variation in this frequency affects the immunisa-

bility. Although a rise in this frequency increases the number of immunised patients up to a certain point, the latter rise is not equally high because the relative number of heterozygous husbands (and Rh negative children) grows even more quickly. A fall in the Rh negative frequency again leads to a rise in the number of homozygous husbands. Without going into any further detail the matter can be illustrated by the following example:

It was established in Table 5 that 41.15 per cent of the Rh negative mothers had given birth to at least two Rh positive children. Including Rh positive mothers the corresponding percentage was 6.08. If now the Rh negative percentage in a population were 9 per cent, 49 per cent of the husbands would be homozygous and 42 per cent heterozygous. If the relative parous group sizes remained constant the number of Rh negative mothers with at least two Rh positive children would be 47.86 per cent and for all mothers the figure would be 4.31 per cent. Though the ratio of the Rh negative percentages was 16: 9, or 1.78, the ratio of those fulfilling the minimum condition for immunisation was only 1.41.

Considering all the different factors affecting the frequency of immunisation, the only conclusion to be drawn is that the frequency of immunisation observed can hardly ever give a true idea of how many of the Rh negative women are immunisable by pregnancies. The probability of immunisation, on the contrary, is independent of many selective factors, and above all of the fertility of the immunised patients. For this reason, a much more realistic basis for the estimation would be obtained from the probability of immunisation calculated for each parous group separately. As a matter of fact, as Rh investigation becomes more and more common an increasing part of the immunisation cases observed are those *first* immunised in connection with the pregnancy at the time of observation. If cases of immunisation observed earlier are excluded from the frequency if the patient has become pregnant again, the result will be a frequency approaching more and more closely the immunisation probability values.

4. The Relative Numbers of the Homozygous Husbands of Immunised Patients

Race [62,38,56] has observed that a greater proportion of the husbands of Rh immunised patients were homozygous than might have been expected from an unselected material. In his material there were 3 homozygotes to each heterozygote, whereas unselected materials give 3 homozygotes to 4 heterozygotes. Roberts [52] has pointed out that a rise of this kind is to be expected since the immunised patients have each been delivered of at least two Rh positive children.

It is, however, obvious that all the different factors mentioned above affecting the frequency of immunisation change, each in its own way, the relative number of homozygous husbands. Consequently, the relative number of the homozygous (and heterozygous) fathers cannot give any idea of any single factor affecting immunisation; it rather reflects the joint effect of many different factors.

Because the husbands of the immunised patients are all Rh positive the most convenient way of examining the changes in the relative number of homozygotes is to start by calculating their relative number from the total of Rh positives in an unselected material. Since homozygotes account for 36 % of the population they represent about 42 % of the Rh positive only (and heterozygotes about 58 %). In Race's material the homozygote ratio rose from 3: 4 to 3: 1, or from 42 % to 75 %.

If, as according to Roberts, the delivery of at least two Rh positive children is regarded as a minimum condition of immunisation, the relative number of homozygote husbands in the Finnish material can be calculated from Table 5. The total number of husbands, then, was 411. 5 $^0/_{00}$, 244.1 of whom or 59.3 % were homozygotes. Race has paid attention to the fact that in a material with large families the rise in the relative number of homozygotes is not as striking as in one with smaller families. If the relative number of homozygotes is calculated from Table 6, representing two extreme Finnish materials in this respect, it is 55.6 % for northern Finland and 64.8 % for Helsinki. Whatever the relative sizes of the different parous groups in a certain material, the relative number of homozygote husbands thus

calculated can never amount to more than 75 %. This is seen from the column of secundiparae in Table 5, where the relative number of homozygote husbands has reached its maximum, exactly 75 %. In all the following groups this percentage declines more radically the more numerous the pregnancies of the mothers in question.

Selection causes remarkable changes in the relative number of homozygote husbands. If the sample is not received until after the birth of several diseased children, the figure rises very radically. If again the investigation is neglected because of an atypical medical history (i.e. a healthy child is born after a diseased one) the number of heterozygote husbands will decrease. If patients with a history of blood transfusion or abortion are included in the material the relative number of heterozygotes will increase.

The husband's genotype is usually determined with four common antisera (anti-C, -D, -E and -c). Because the determination is based on a guess with a known probable error, a certain part of the homozygotes are actually heterozygotes and vice versa. In the following ten of the most common genotypes are presented according to Race[53]. According to the results obtained from genotype determination, those on the left are regarded as homozygotes and those on the right as heterozygotes.

C+ D+ E− c− C+ D+ E+ c+			C+ D+ E− c+ C− D+ E+ c+		
CDe/CDe	17.7 %		CDe/cde	32.7 %	
CDe/cDE	11.9 %	29.6 %	cDE/cde	11.0 %	43.7 %
CDe/cde	0.8 %		CDe/cDe	2.2 %	
Cde/cDE CDe/cdE	1.3 %	2.1 %	cDE/cDE cDE/cDe	2.7 %	4.9 %

Thus the real homozygotes are $29.6 + 4.9 = 34.5$ % and real heterozygotes $43.7 + 2.1 = 45.8$ %. Accordingly $100 \cdot 2.1 : 45.8 = 4.6$ % of the heterozygotes have been erroneously regarded as homozygotes, while $100 \cdot 4.9 : 34.5 = 14.2$ % of the homozygotes similarly have been taken for heterozygotes.

Let us assume that among the husbands of the patients with at least two positive children 59.3 % are homozygotes and 40.7 % heterozygotes, as the case is in Table 5. Of the former 14.2 %,

or 8.4 would have been falsely regarded as heterozygotes and of the latter 4.6 %, or 1.1 as homozygotes. If the genotype determination of this material had been carried out in the usual way, the result would have been as follows:

$$\text{homozygotes} = 59.3 - 8.4 + 1.1 = 52.0\ \%$$
$$\text{heterozygotes} = 40.7 - 1.1 + 8.4 = 48.0\ \%$$

Thus genotype determination based on a guess seemingly diminishes the relative number of homozygous husbands very much. If it is assumed e.g. that in a certain material 75 % of the husbands are taken for homozygotes in this method of genotype determination, the real number of homozygous husbands can be calculated from the figures given above. If we denote this value by x, the real number of heterozygotes is 100 — x. x can then be calculated from the following equation:

$$x - \frac{14 \cdot 2}{100} \cdot x + \frac{4 \cdot 6}{100} \cdot (100 - x) = 75 \quad x = 87.1\ \%$$

The proportion of homozygous and heterozygous husbands in this material, accordingly, is 87.1 : 12.9, or 6.75. Because this proportion is 3: 4, or 0.75 in an unselected material, the proportion here has increased 9- fold in favour of homozygotes. When determining the genotypes of the husbands of immunised patients belonging e.g. to the groups CDe/cde or CDe/cDe the probable error has risen from 6 % in an unselected material to

$$\frac{9 \cdot 94}{6} : \frac{94 + 9.6}{9 \cdot 6}, \text{ or nearly } 34\ \%.$$

When estimating an HDN family's likelihood of having an Rh negative child in the future it is, however, better to calculate the error in genotype determination with regard to the number of (Rh positive) children previously born to the family. As, however, this is outside the scope of the present investigation, reference only is made to an earlier investigation into the matter [46].

CHAPTER II

IMMUNISATION THROUGH BLOOD TRANSFUSIONS

1. Frequency of Immunisation

Up to 1947 Rh immunisation by blood transfusions was considered fairly unusual. Immunisation through pregnancy was known to occur once in 200—300 births. As, in addition, haemolytic transfusion complications giving clinical symptoms and caused by Rh immunisation were found in few cases only, it was natural that Rh immunisation was considered rare, whether pregnancy or blood transfusion was the fundamental cause.

However, several investigations published in the last five years have shown that a considerable number of people are capable of forming Rh antibodies as a result of Rh positive blood transfusion. Among these investigations the work of Diamond [21] deserves special mention, having been based on extensive material, in which he found that nearly a half of 200 Rh negative subjects (92, i.e. 46 %) who had received transfusions during the war became immunised. In a similar material Hattarsley [30] found 11 out of 20 negative subjects immunised, and Diamond [21] found that more than half of 22 unmarried transfused women he examined were immunised.

The immunising influence of transfusions was still more distinctly seen in investigations where Rh positive blood was transfused into Rh negative volunteers. As the different investigators have employed different immunisation methods the results are not fully comparable. Wiener and Sonn-Gordon [77] immunised five out of 9 persons with two 4 ml injections given at an interval of 4 months. Diamond [22] immunised 45 % of 35 patients by giving them first a fairly large injection and later on repeated small injections.

A highly illustrative picture of the development of immunisation induced by blood injections is supplied by Wiener's [78] series of experiments. He injected a total of 47 persons with the equi-

valent of 2—4 cc red cells at an interval of four months. A good third (18) were immunised with two injections, 23 of those who had still failed to form antibodies were given another injection, on which 6 formed antibodies. 15 of the remainder received yet another injection, resulting in 3 more immunised. One only of the ten persons who received a fifth injection became immunised. The number immunised, therefore, totalled 28. Allowing for the fact that 10 test subjects evidently did not receive the necessary number of injections, it can be said that a good two-thirds of people can be immunised even by such small, repeated blood injections.

This investigation is supported by Waller's [63] series of experiments in which the blood injections were given at six-weekly intervals. Three out of ten test subjects were immunised after three injections, one more was immunised after a fourth, three more after a fifth, and after six injections all but one had formed antibodies.

By way of a summary of these investigations it may be stated that

— the majority of Rh negative persons become immunised as a result of repeated blood injections or transfusions,

— increasing the number of injections may considerably raise the total of the immunised. A small number of people, however, appear to be incapable of forming Rh antibodies.

Our knowledge of how large a proportion of the population is capable of forming Rh antibodies as a result of blood injections may be considered adequate for judging human immunisability against Rh antigen. The question as to how blood injections or transfusions given to a female patient eventually affect the child born by the patient subsequently is not easy to answer and the problem cannot be studied by means of organised experiments. The question must be reckoned of primary importance from both the practical and the theoretical point of view. It must be considered possible that a comparison of cases of pure pregnancy immunisation with cases in which immunisation has been started by transfusion may reveal differences of importance to our efforts at ascertaining more about the mechanism of pregnancy immunisation.

Before embarking on a discussion of the influence of trans-

fusion on babies born subsequently, an account will be given of the material investigated by the present author, covering transfused female patients of fertile age. Rh research has become so generalised in recent years in the majority of countries that it will not be easy in the future to collect a relatively unselected material of transfused patients in which the Rh groups have been disregarded. The publication of such a material may be considered justifiable on these grounds although, because of its limited scope, it will permit no far-reaching conclusions one way or another.

2. Follow-up Examination of Transfused Female Patients

a) *Purpose of the work*

The purpose of the work has been to examine the following points:

— The immunisation frequency against D and possibly against other antigens as well.

— The fate of post-transfusion Rh positive infants.

— The effect of post-transfusion Rh positive infants on the frequency of immunisation.

b) *Material*

In order to collect the material, the 1943—1948 journal card indices of the Women's Clinic, Institute of Midwifery (obstetric and gynaecological cases) and Finnish Red Cross Hospital (surgical cases) were scrutinised. From them were selected the female patients aged 16—40 who had received one or several blood transfusions. As the transfusion entries, in the earlier years in particular, were very incomplete a considerable number of patients transfused at these hospitals was doubtless excluded for this reason.

A total of 1,000 such patients were sent an invitation to submit a blood sample. The letter mentioned briefly the purpose of the investigation, but no reference was made to any possible harmful effect of blood transfusions on the patient or her child. In the cases where the letter was not returned by the postal authorities

or the patient failed to turn up, another letter was mailed at monthly intervals, four times in all. In some cases where the letter was returned the patient's address was traced from the town inquiry office.

A total of 220 letters were returned because of unknown address, death of the patient, emigration etc. Hence it may be considered that 780 patients were informed of the examination arranged. Of them 509, i.e. 65.3 %, turned up for examination.

Each patient found to be Rh negative was called for a new examination. In addition, as far as possible, the husbands, living children and blood donors of these patients were examined.

From those resident in Helsinki the blood samples were taken at the Red Cross Blood Service laboratory; with those dwelling in the countryside, samples were taken by the local public health nurses or midwives. At the same time as detailed a medical history as possible was noted for each patient and compared with the data entered in the journal. All the serological investigations were effected by the Blood Grouping Laboratory of the State Serum Institute between Oct. 1, 1950 and June 1, 1951.

c) *Evaluation of the tests, and nomenclature*

It is often difficult to judge from a purely serological examination whether or not the subject is immunised. The simple solution adopted in the following was that every patient whose serum showed a positive reaction to the indirect Coombs test was considered immunised irrespective of what the result had been with other methods of investigation (however, the results yielded by trypsinated red cells concurred with those of Coombs test).

All the cases where agglutination was obtained with anti-D serum were considered Rh positive. The D negative subjects who nevertheless had C antigen and gave a positive result with albumin-agglutinating anti-D serum by Coombs technique, were also considered Rh positive.

For the sake of uniformity, Fisher and Race's [56] nomenclature has been employed exclusively in the present work. For the benefit of readers who are accustomed to Wiener's nomenclature, the following table gives the most usual Rh antigens and antibodies in both nomenclatures.

TABLE 8

Antigens			Antibodies					
Fisher-Race	Wiener	Fisher-Race Wiener	Anti-D Anti-Rh_0	Anti-C Anti-rh′	Anti-E Anti-rh″	Anti-d Anti-Hr_0	Anti-c Anti-hr′	Anti-e Anti-hr″
cde	rh		—	—	—	+	+	+
Cde	rh′		—	+	—	+	—	+
cdE	rh″		—	—	+	+	+	—
CdE	rh_y		—	+	+	+	—	—
cDe	Rh_0		+	—	—	—	+	+
CDe	Rh_1		+	+	—	—	—	+
cDE	Rh_2		+	—	+	—	+	—
CDE	Rh_z		+	+	+	—	—	—

Rh antigens, antibodies, and their interactions in Fisher and Race's and Wiener's nomenclatures.

The genotypes have been entered so that all the antigens are visible, and the most frequent of the various genotype possibilities has been selected. However, if a study of the genotypes of the patient's children has made it evident that the father in fact belongs to another less frequent combination his genotype has been amended accordingly.

d) *Results*

On the basis of their Rh groups, the distribution of the 509 patients examined was as follows:

TABLE 9

cde/cde	64			
Cde/cde	5	*Rh-negative*	69	(13.6 per cent)
D-positive	435			
CD^ue/cde	5	*Rh-positive*	440	(86.4 per cent)

The distribution of Rh-groups in 509 patients with a history of blood transfusion.

None of the examined belonged to group cdE/cde or cD^uE/cde.

The AOB groups of the patients were examined in 506 cases; in 3 cases the examination was omitted by error. The distribution of the AOB groups was as follows:

TABLE 10

A	228	(45.1 per cent)
O	152	(30.0 per cent)
B	83	(16.4 per cent)
AB	43	(8.5 per cent)

The distribution of AOB groups in 506 patients with a history of blood transfusion.

The following table gives the results of the antibody investigation of the serum of all the patients.

TABLE 11

D positive	440	
anti-c immunised ..	3	(0.7 per cent)
D negative	69	
anti-D immunised ..	35	(51 per cent)

Immunisation frequency in 509 patients with a history of blood transfusion.

In addition, side by side with anti-D, weak anti-C antibodies were found in five cases. In no case were any other Rh antibodies found, any more than anti-Kell, anti-Lewis, etc., although the corresponding antigens were contained in the control red cells.

The following table gives the numbers of bottles received by immunised and non-immunised Rh negative patients.

TABLE 12

	Number of patients		
Number of bottles	Immunised	Not immunised	Total
1	5	5	10
2	17	20	37
3	3	4	7
4 or more	10	5	15
Total	*35*	*34*	*69*

Number of bottles transfused to 69 patients with a history of blood transfusion.

It may be estimated that 15 % of the patients receiving one bottle had been given Rh negative blood. These patients totalled ten only. Among patients who had received two bottles 2.25 % may be estimated to have received only Rh negative blood. As these patients totalled 37 it must be considered probable that 2—3 patients had received exclusively Rh negative blood. It may be mentioned that in all the cases but one (Case No. 11), in which the serial numbers of the bottles were entered in the journal and the donor had been examined either before or afterwards, one of the two donors at least was Rh positive.

61 patients had received a single transfusion, the rest two or more. If two or more transfusions took place within the same week they were considered as a single transfusion.

The immunised Rh negative subjects had received an average of 1.17 blood transfusions and an average of 2.51 bottles of blood. For the other Rh negatives the corresponding figures were 1.11 and 2.35. Comparison with the figures of the Rh positive group shows that the latter had received an average of 2.22 bottles in an average of 1.21 transfusions. These figures, too, indicate that the Rh negativity of the patient has not prevented the administration of transfusions on the same scale as to other patients.

The frequency of abortion is high both with the Rh negative and Rh positive since abortion had in many cases constituted the indication for blood transfusion in the material. Half the former (35) had one or several abortions, as did 75 % (255) of the latter. The total abortions and deliveries of Rh negative patients can be seen from the following table:

TABLE 13

Number of abortions	Number of deliveries									
	0	I	II	III	IV	V	VI	VII	VIII≤	Total
0	3	12	10	4	3	—	—	1	1	34
1	2	4	5	3	5	1	—	—	1	21
2	—	3	2	3	—	1	—	—	1	10
3	—	1	1	—	—	—	—	—	—	2
4	—	—	—	1	—	—	1	—	—	2
Total	*5*	*20*	*18*	*11*	*8*	*2*	*1*	*1*	*3*	*69*

Number of deliveries and abortions of 69 Rh negative patients with a history of blood transfusion.

If each abortion, delivery and blood transfusion is considered an immunisation stimulus the distribution of the stimuli of the Rh negative patients between the immunised and non-immunised was as follows:

TABLE 14

	Number of stimuli								
	1	2	3	4	5	6	7	8 or more	Total
Immunised	2	4	6	5	9	4	2	3	35
Not immunised	—	9	6	7	2	4	1	5	34

Number of stimuli of 69 Rh-negative immunised and non-immunised patients with a history of blood transfusion.

The stimuli totalled 165 (average 4.7) with the immunised and 143 (average 4.2) for the non-immunised.

A sample was obtained from 53 husbands of Rh negative patients. Seven husbands refused to give a sample, 6 patients were unmarried and two divorced (the residence of the ex-husband was unknown), and the husband of one patient had died. The following table gives the AOB and Rh groups of the husband, probable genotype with regard to D, and AB compatibility and incompatibility compared with the wife's group.

TABLE 15

	Blood group of husband									
	A	O	B	AB	Rh+	Rh—	Homoz.	Heter.	Compat.	In-compat.
Immunised ...	17	4	4	1	26	1	11	15	15	12
Not immunised	12	7	7	1	21	5	10	11	16	10
Total	29	11	11	2	47	6	21	26	31	22

The distribution of AOB and Rh groups, homozygoty and heterozygoty, and compatibility and incompatibility of 53 husbands to immunised and non-immunised Rh-negative patients with a history of blood transfusion.

The Rh negative patients had a total of 223 pregnancies, their distribution being:

TABLE 16

Group of children	Number of pregnancies
Rh positive	88
Rh negative	45
Not determined	37
Stillbirth	9
Abortion	54
Total	*223*

Number of Rh positive and negative children, stillbirths, and abortions of 69 Rh-negative patients with a history of blood transfusion.

If the unexamined children are excluded the number of negative children (45, i.e. 36 %) is approximately what was to be expected on the basis of the genotypes of the husbands examined: 21 of the husbands were homozygous, 26 heterozygous and 6 Rh negative; hence, 36 % of the children should be negative.

The following table gives the pregnancies and transfusions of the Rh negative patients, the type of antibody observed and the fate of the infant. The table is divided into three sections: in the first, all the patients have given birth to an Rh positive child since the transfusion; in the second, all the patients are Rh immunised but have not given birth to an Rh positive child since the transfusion; and in the third, the patients are neither immunised nor have they given birth to positive children since the transfusion. (See Table 17.)

Cases 1 and 32 (J. No. 80 and 711) of the table had evidently been immunised prior to the transfusion by repeated pregnancies. In addition, the latter case received Rh negative blood; hence, the transfusion cannot be responsible for the immunisation.

TABLE 17

Casuistic data of 69 Rh negative and 3 Rh positive patients with a history of blood transfusion.

I

Cases with an Rh positive child delivered after blood transfusion.

Abbreviations:

Rh +	Rh positive child
Rh —	Rh negative child
Ab.	Abortion
St.	Stillbirth
1	Healthy or subclinical HDN
2	HDN recovered
3	Died

N:o	Group of mother	Type of antibody	Titre in albumin	Months since Rh-positive stim.	Number of bottles transfused	Group of father	Consecutive order of pregnancy I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
80	AB	cde	D	32	35	3	O CDe/CDe	Ab.	?[1]	?[3]	Ab.	?[3]	?[3]	?[3]	?[3]	Tr. Rh+[1]	St.		
85	O	cde	D	8	0	2	A CDe/CDe	?[1]	Ab.	Tr. Rh+[1]	Ab.	Rh+[2]							
94	A	cde	D	128	0	2	A CDe/cde	Tr. St.	Rh+[1]	Rh+[3]									
106	O	cde	D	32	18	2+2	A CDe/cDe	Rh+[1]	Rh+[1]	Tr. Rh+[1]	Tr. St.								
114	O	cde	—		48	2	A cDE/cde	Tr. Rh+[1]											
166	A	cde	D	512	0	2	A cDE/cde	Rh—	Tr. Ab.	Rh+[3]									
285	O	cde	D	128	0	2+?	O CDe/CDe	Rh+[1]	Rh+[1]	?[1]	Rh+[1]	?[1]	?[1]	Rh+[1]	Rh—	Tr. ?[1]	Tr. Rh+[3]	St.	St.
291	A	cde	D	16	0	2+?	A cDE/cde	Tr. Rh+[1]	Ab.	Tr. Rh+[1]	Rh+[1]	Rh+[2]							

291	A	cde	D	16	0	2+?	A	cde			Rh+1	Ab.	Rh+1	Rh+1	Rh+1					
384	AB	cde	D	256	0	1	A	cDE / cDe			Rh+1	Tr. Ab.	Rh+2	Rh+2						
407	B	cde	D	128	0	2+2	A	cDe / cde			Tr. Ab.	Rh—	Tr. St.	Rh+2 Rh+2						
509	O	cde	D	8	12	4	?	?			Rh—	Rh+1	Rh+1	Rh—	Rh+1	Tr. Ab.	Rh—	Rh+2	Rh+3	
672	B	Cde	—		30	2	A	CDe / CDe			Rh+1	Rh+1	Rh+1	Tr. Ab.	?3	Rh+1				
678	O	cde	D	64	24	2	?	?			Rh+1	Ab.	Tr. St.	Rh+1						
696	O	Cde	—		16	4+?	B	CDe / cde			Tr. Rh+1	Tr. Rh+1								
765	A	cde	—		13	2+?+?	AB	CDe / cDE			?3	Ab.	Tr. Rh+1	Tr. Ab.	Tr. Rh+1					
776	AB	cde	D	32	9	1	A	CDe / cDE			Tr. Rh+1	Rh+1								
855	A	cde	D	1	24	2	?	?			Rh+1	Tr. Ab.	Ab.	Rh+1						
15	A	cde	C+D	128	0	5+6	O	CDe / Cde	Tr.	Tr.	?3	?3	Rh—	Rh+2						
(623)	A	CDe / CDe	c	8	18	1	AB	CDe / cde			c+1	c+1	Ab.	Tr. c+1	c+1	c—1	c—1	c—1	c+1	
(792)	O	CDe / CDe	c?	1	27	2	O	CDe / CDe			c—1	Tr. c—1								
(746)	O	CDe / CDe	c	1	24	2	O	cde / cde			c+1	Tr. ?3	c+1							

II

Rh immunised patients with no Rh positive children after the transfusion.

N:o	Group of mother	Type of antibody	Titre in albumin	Months since Rh-positive stim.	Number of bottles transfused	Group of father	Consecutive order of pregnancy						
							I	II	III	IV	V	VI	VII
7	A	cde	D	1	30	2	?	?	Rh+[1]	Tr. Ab.			
67	A	cde	D	4	52	2	B	CDe/cde	Rh+[1]	Tr. Rh+[1]			
70	O	cde	C+D	8	52	2	A	CDe/cde	Rh—	Tr.	Rh—		
100	O	cde	D	4	60	2	?	?	Tr. ?[1]				
151	O	Cde	D	1	73	2	B	cDE/cde	Rh+[1]	Ab.	Ab.	Tr. Ab.	
205	A	cde	D	32	54	2	B	CDe/cde	Rh+[1]	?[1]	Rh+[1]	Rh—	Ab.	Tr. Ab.
271	B	cde	C+D	64	42	2	B	CDe/cde	Tr. Rh+[1]	Rh—			
315	B	cde	D	16	27	1	A	cDE/cde	Tr. Rh+[1]	Rh—			
335	A	cde	D	16	12	2	A	CDe/cde	?[1]	Tr. Ab.	Ab.		
368	B	cde	D	8	45	2	O	CDe/CDe	Rh+[1]	Rh+[1]	Tr. Ab.		

368	B	cde	D	8	45	2	O	$\frac{CDe}{CDe}$		Rh+[1]	Rh+[1]	Tr. Ab.				
525	B	cde	D	128	66	2	?	?		?[1]	Tr.					
622	A	cde	D	128	30	3+1	?	?		Rh+[1]	Rh+[1]	?[1]	Tr.	Ab.	Tr. Ab.	
711	A	cde	D	16	28	1	A	$\frac{CDe}{cDE}$		Rh+[1]	?[3]	Tr. Rh+[3]				
37	A	cde	C+D	8	60	2	O	$\frac{CDe}{cde}$		?[1]	?[1]	Rh+[1]	Tr. Rh—			
67	A	cde	C+D	16	14	2	AB	$\frac{CDe}{cde}$		Rh+[1]	Rh+[1]	Rh+[1]	Tr. Rh+[1]	Ab.		
82	O	cde	D	1	30	1	?	?		?[1]	?[1]	Tr. ?[1]				
11	O	cde	D	512	72	4	—		Tr.							
16	A	cde	D	4	66	4	?	?		Rh+[1]	Tr.					
31	O	cde	D	32	60	3	—		Tr.							
29	A	cde	D	8	57	3+4	A	$\frac{cde}{cde}$		Rh—	Rh—	Rh—	Rh—	Tr.	Tr.	
38	A	cde	D	4	37	4	A	$\frac{CDe}{cde}$		Rh—	?[1]	St.	Tr.			

III

Non-immunised patients with no Rh positive children after the tranfsusion.

N:o	Group of mother	Type of antibody	Titre in albumin	Months since Rh-positive stim.	Number of bottles transfused	Group of father	Consecutive order of pregnancy I	II	III	IV	V	VI	VII	VIII	IX	X
1	A	cde		12	2	A	$\frac{CDe}{CDe}$ Rh+[1]	Tr. Ab.	Ab.							
4	O	cde		57	2	O	$\frac{CDE}{cDE}$ Rh+[1] Tr.	Rh+[1]	Tr. Ab.							
51	O	cde		60	?	?	?	?[1]								
57	O	cde		51	?	A	$\frac{CDe}{cde}$ Rh+[1] Tr.	Tr. Rh+[1]	Rh—							
71	A	cde		49	2	A	$\frac{CDe}{cde}$ Rh—									
203	O	cde		24	2	A	$\frac{cDe}{cde}$ Rh+[3]	Tr. Ab.	Rh—	Ab.						
233	A	cde		25	4	O	$\frac{CDe}{CDe}$ Rh+[1] Tr.	Rh+[1]	Tr. Ab.							
304	A	cde		40	2	A	$\frac{CDe}{cde}$ Rh— Tr.									
355	O	cde		39	2	?	? Ab.									
360	O	cde		44	1	B	$\frac{cDE}{cde}$ Rh+[1] Tr.	Tr. Ab.								
377	B	cde		48	3	A	$\frac{CDe}{cDE}$ Ab.									
388	A	cde		34	2	A	$\frac{cde}{cde}$ Rh—	Tr. Ab.	Ab.							
428	A	cde		72	3	A	$\frac{cde}{cde}$?[1]	?[1]	Rh—	Tr. Ab.	Rh—					

458	A	Cde	60	2	A	CDe / cDE		Tr. Rh+[1]	Rh+[1] Tr.									
467	O	cde	60	2	?	?	Ab.											
505	O	cde	70	3	O	CDe / cde	Ab.		Tr. Rh+[1]	Rh+[1]								
560	A	cde	60	2	O	cde / cde	Ab.	Ab.	Rh—	Rh—	Rh—	Rh—	Rh—	Rh—	Tr. Ab.	Ab.		
708	A	cde	30	1	B	CDe / cde	Rh+[1]	Tr. Rh—										
724	B	cde	12	?+?	A	cDE / cde	Rh—	Rh+[1]	Rh+[1]	Ab.	Rh—	Tr. Ab.	Tr. Rh—					
736	AB	cde	24	?+2	O	cde / cde	Tr. ?[1]	Tr. Rh—										
769	O	cde	30	2	?	?	Ab.	Tr. ?[1]										
777	O	cde	34	3	?	?	Tr. Rh+[1]											
812	A	cde	30	2	?	?	Rh—	Rh+[1]	Rh—	Tr. Ab.								
825	B	cde	36	2	B	CDe / cDE	Rh+[1]	?[1]	Ab.	Ab.	Tr. Ab.							
819	O	Cde	30	1	?	?	Tr. Ab.											
8	AB	cde	37	1	A	CDe / CDe	Rh+[1]	Ab.	Rh+[1]	Tr. Ab.	Ab.							
21	AB	cde	60	2	?	?	Rh—	Rh—	Rh—	Rh—	Tr. Rh—							
30	O	Cde	60	2	A	CDe / cDE	Tr. ?[1]											
50	O	cde	46	1	A	CDe / cDE	Tr. ?[1]											
68	A	cde	31	2	B	CDe / cde	Tr. Rh+[1]											

As to the fate of the children born after the transfusion, the material must be said to have »shrunk» regrettably. 34 patients had had no pregnancies at all since the first transfusion. The prognosis of the post-transfusion pregnancies of 35 patients, including 20 immunised, can be seen from the following table. The fate of the first post-transfusion Rh positive child only is given in brackets.

TABLE 18

	Immunised	Not immunised	Total
Rh positive children:	24 (18)	5 (4)	29 (22)
Healthy or subclinical HDN	5 (5)	5 (4)	10
HDN. Recovered	8 (5)	—	8
HDN. Died	6 (3)	—	6
Stillborn	5 (2)	—	5
Rh negative children	5	8	13
Abortion	6	6	12
Total	*35*	*19*	*54*
No pregnancies	15	19	34

The outcome of pregnancies after blood transfusion in 69 Rh-negative patients with a history of blood transfusion.

e) *Technique*

The blood samples taken from the veins of the transfused patients, 1—4 days old at the time of examination, were centrifuged and the serum was separated. A physiological saline suspension, 2—3 % by visual estimation, was prepared from the red cells.

All the samples were first examined with a human anti-D serum agglutinating in physiological saline; its titre was 1: 32 or higher. If the result was negative the sample was re-examined with the same serum and, in addition, with another anti-D serum and with anti-C, anti-E and anti-c sera. In all cases where an indefinite result was obtained with anti-D serum the sample was examined by the Coombs technique using strong, albumin-agglutinating anti-D serum. The same examination was made if C was found in the red cells but D was missing.

The sera of all the samples were examined with two or three different control red cells by four different techniques: saline agglutinins in the usual way, albumin agglutinins with red cells suspended in albumin, with trypsinated red cells and by the Coombs

technique. The arrangement of the experiments can be seen from the following table:

TABLE 19

	Patients red cells + anti-D serum	Patients serum + CDe/cDE in saline	cde/cde in saline	CDe/cDE in album.	cde/cde in album.	CDe/cDE trypsinated
Tube	1	2	3	4	5	6

The technique used in the investigation of patients with a history of blood transfusion.

After checking the saline agglutinins in tubes 2 and 3, the indirect Coombs test was effected on the same tubes.

In addition to the six Rh antigens, the cells employed always had the following agglutinogens: M, N, S, Kell, Le^a, Le^b and P. Unfortunately C^w control cell was not available. The cells had not been examined for Duffy, Lutheran and Levay groups.

The sera of all the patients that had given agglutination with control cells in the first examination were re-examined, in order to find out the antibody type, with the following cells: CDe/cDE, CDe/CDe, Cde/cde, cdE/cde, cDE/cDE and cde/cde. Both cells suspended in albumin and cells treated with trypsin were employed in this examination. In addition, in certain cases, the indirect Coombs technique was employed to determine the antibody type.

The family members of Rh negative patients were examined with anti-C, anti-D, anti-E and anti-c sera. In addition, Coombs test was carried out with the patient's serum against the red cells of the husband and the children, provided they were compatible in their AOB groups.

The technique employed in the different tests was mainly that described in Medical Research Council Memorandum No. 19.

Rh groups:

The patient's red cells are washed once and a suspension, approx. 2 % by ocular estimate, is prepared with physiological saline solution. Approx. 1/50—1/100 cc of test serum and the same quantity of the red cell suspension to be examined are dropped into a 50 × 4 mm tube by a graded Pasteur pipette fitted with a rubber cap. The racks with the tubes are placed in a + 37° C incubator for an hour and a half. Readings are taken microscopically using a Pasteur pipette and object glass.

Saline agglutinins:

A drop of the patient's serum and a drop of once washed red cells, diluted with physiological saline to approx. 2 %, are dropped into a 50×8 mm tube. The rack with the tubes is placed in a +37°C incubator for 1 ½—2 hours, and readings taken macroscopically. If no agglutination is found the indirect Coombs test is made using the same cells (see below).

Albumin agglutinins:

1. A whole day's supply of control red cells are washed once with physiological saline solution, centrifuged, and the packed red cells are diluted to a 2—3 % suspension with 20 % bovine albumin (Armour Laboratories). The test is effected in the same way as for the examination of saline agglutinins, only smaller tubes (50×4 mm) and smaller volumes are used. Readings are taken microscopically using a Pasteur pipette and object glass.

2. A whole day's supply of control red cells are washed in the same way as above. Solution containing 10 mg trypsine in 12 cc physiological saline buffered to pH 8.4 with phosphate buffer is added to the tube, which is placed in a + 37° C water bath for 30 min. The tube is shaken 2—3 times in the water bath and the red cells centrifuged and washed once with a liberal quantity of physiological saline solution. The red cells are diluted with saline to a 2—3 % solution. The test itself is carried out in the same way as with cells suspended in albumin.

3. Indirect Coombs test: The red cells used for the examination of saline agglutinins are washed three times with approx. 30 volumes of physiological saline solution. A drop of suitably diluted (see below) anti-human-precipitin serum and a drop of red cells in 2 % dilution are dropped onto an opaque glass plate. The plate is rocked for 8—10 minutes and the readings taken macroscopically by transillumination.

The anti-human-precipitin serum was first prepared by using aluminium-precipitated human serum, according to Proom, injected intramuscularly into a rabbit two or three times at weekly intervals. Later, human gamma globulin according to van Loghem was employed for immunisation. The immune serum obtained was examined with fully-sensitised red cells and absorbed, to remove unspecific agglutinins, with A, B and O red cells washed 6 times, or by heating the serum for 2 hours at +65°C. The dilution, on the basis of the investigation carried out, was generally effected with 150—200 parts of physiological saline solution, to prevent prozone effect.

Determination of titre:

The titre is determined separately with red cells suspended in physiological saline, in albumin, and with those treated with trypsin. Two drops of physiological saline solution are dropped with a Pasteur pipette into each of the requisite number of 50×8 mm tubes. Using the same pipette, two drops of the serum to be examined are dropped into the first saline tube, mixed, and 2 drops of the serum thus diluted are transferred to next tube,

and so on. The test is carried out with the different red cells according to the description above.

f) *Conclusions*

If the distribution of Rh groups in the material is compared with the investigations previously effected in Finland the D^u positive must be included in the Rh negative group. Hence, the material includes 14.5 % of Rh negative patients, or slightly less than is indicated by the study of Finnish blood donors in 1947 (15.7 %) [45]. In this connection it may be mentioned that the Maternity Advisory Clinic patients in Helsinki, of whom a total of 12,146 were examined in 1949—1951, included 1,776, i.e. 14.62 % whose reaction was negative to anti-D serum.

Of the C positive but simultaneously D negative patients, on the basis of the Coombs test effected, half had to be considered positive, and half negative. The size of the group, 10 out of 509, i.e. 2 %, is somewhat smaller than the 2.9 % established in Finland previously. The difference is probably primarily due to the fact that the anti-D sera employed in the present investigation were stronger than those in use a few years ago.

For the sake of comparison an examination was made of 100 C positive but D negative blood donors. To the indirect Coombs test 49 were positive, with strong albumin-agglutinating anti-D serum; 51 gave a negative result.

Due to the limited size of the material, hardly any conclusions can be drawn regarding the prognosis of the Rh positive children of immunised mothers. They totalled (including stillbirths) 24, of which 11 died.

On the other hand it is evident that immunised patients had considerably more Rh positive children after the transfusion than the non-immunised: the former group had 24 children and the latter only 5. It can be seen that the patients who gave birth to Rh positive children after the transfusion totalled 18 in the material, including 14 immunised. Of the 51 patients who had not given birth to Rh positive children since the transfusion, 21 only were immunised.

From this it seems evident that at least a number of the patients who had not been immunised had nevertheless been sensibilised by the transfusion received. The absence of manifest immunisation, accordingly,

would be due to the fact the patients had not received an immunising stimulus (an Rh positive child or blood transfusion) since the sensibilisation.

A study of how many of the post-transfusion Rh positive children of the transfused mothers were affected with HDN shows that there were half of them (9); the other half (9) were healthy or had the disease in such a mild form that it was not diagnosed. Two of the three patients with anti-c antibodies had given birth to a c positive child since the transfusion. In neither of the two children was HDN diagnosed.

Summary of the results of the follow-up examination

— The material comprised 509 female patients of 15—40 who had been given one or several blood transfusions.

— 69 (13.6 %) were Rh negative. 5 belonged to group CD^ue/cde.

— 35 of the Rh negative (51 %) were immunised against D antigen and 5 of these additionally against C antigen. None of those belonging to group CD^ue were immunised. Three of the Rh positive (0.74 %) had anti-c antibodies. No other antibodies could be demonstrated.

— A total of 18 Rh negative patients had given birth to one or several Rh positive children after the blood transfusion. 14 of these patients had become immunised. The frequency of immunisation was considerably lower in the cases where the patient had had no post-transfusion Rh positive children. They totalled 51, including only two-fifths (21) immunised. The author considers it probable that a remarkable number of the non-immunised patients have in fact become sensibilised, in which case the birth of an Rh positive child would manifest the immunisation serologically and in many cases also clinically.

— 24 of the total of 29 post-transfusion Rh positive children were born to Rh immunised mothers. 11 of them died of HDN. Taking only the first Rh positive post-transfusion child into consideration, half the children of 18 mothers had manifest HDN and the other half were healthy or had the disease in subclinical form.

3. Effect of Blood Transfusions on HDN Frequency

The proportionate number of patients of an HDN material who have received blood transfusions supplies indirect evidence of the proportion of transfused patients who give birth to diseased infants. Of Pickles's [49] material of 78 cases, 8 had received transfusions (10 %); the corresponding figures for Mollison's [43] material of 1947—1948 were 20 out of 66 (30 %), for Hellman and Vosburgs's [31] 7 out of 27 (26 %), for Potter's [54] 8 out of 122 (6.5 %), Davidsohn's [13] 12 out of 73, Hartmann's [28] 63 out of 520 (12.1 %), and for the present author's material [47] of 1948—51 35 out of 282 (12.5 %).

Discombe and Hughes [24], studying the transfusion frequency of the Rh immunised, gave the transfusion frequency for the total material. In their material 8 of the 25 immunised had received a blood transfusion; the transfusion frequency for the total material was 2 % only. It is regrettable that the incidence of transfusion for parturients is not more generally known: with its aid it would be possible to obtain a more accurate picture of how many of the infants of mothers who have received transfusions really contract a haemolytic disease. As Discombe and Hughes's material is too limited to permit of conclusions, the matter may be illustrated with the following example:

Let us assume that the HDN frequency in a material is 1: 250 births, blood transfusion frequency of the immunised 20 % and blood transfusion frequency of the whole material 2 %. Let us also take the total number immunised as 100 (20 transfused, 80 others). The material will then total

$$\frac{100 \cdot (250 \cdot 80)}{98} = 20{,}408.$$

Blood transfusions have been given in 408 cases, including 15 % or 61 Rh negative. We know that 60 % of the children of Rh negative mothers are Rh positive, and hence 37 of the transfused mothers in the delivery studied here have given birth to an Rh positive child. As 20 of them were diseased the disease percentage in this example, computed from the number of Rh positive children, is 57 %.

In this connection it may be pointed out that transfusions of Rh positive blood to an Rh negative parturient increase considerably the number of the immunised, although the effect of transfusion as an immunisation stimulus may be exactly the same as that of an Rh positive foetus. Assuming two Rh positive stimuli to be the minimum requirement for immunisation the total number of parturients meeting the minimum requirement in Table 5 is practically doubled if every one of them has received one transfusion of Rh positive blood. This would doubtless become evident if it were possible to study the genotypes of the husbands of the patients immunised by transfusions: the homozygote proportion would certainly not be nearly as large as in cases of pure pregnancy immunisation.

Although the above may be of importance as a factor increasing the proportion of transfusion immunisations, it does not explain the high blood transfusion frequency in immunisation cases. Firstly, the percentages of all the materials are exceedingly high compared with the blood transfusion frequency which, in the Northern Countries at least, hardly exceeds the figure given by Discombe and Hughes. Secondly, it is remarkable that, should a patient who has received transfusions give birth to HDN children, the very first positive child born after a transfusion will contract the disease. In the present author's material [47] of 38 HDN mothers who had received a blood transfusion the first post-transfusion positive child was diseased in 36 cases; in two cases only was the diseased child preceded by a »healthy», Rh positive infant. From these two points alone it may be considered as certain that transfusing the mother multiplies the HDN frequency compared with the HDN frequency caused by pregnancy immunisation alone.

4. Effect of a Blood Transfusion Given to the Mother on the Prognosis of the Child

Levine and Waller [41] were the first to devote attention to the effect on the prognosis of the child of a blood transfusion given to a mother whose first child contracted the HDN. Of a total

of 19 cases one only showed a slight form of HDN, six a severe form of the disease, and in 12 cases the disease was fatal to the child or the foetus. Out of Chown's [9] 14 cases (instead of a blood transfusion some of the mothers had an abortion in the medical history), 8 died and two had no manifest disease. The following table gives the prognosis of post-transfusion (Rh positive) children according to the present author's material [47].

TABLE 20

	Consecutive order of the sick child			
	I	II	III	Total
Recovered	18	6	2	26
Exchange transfusion	3	3	2	8
Blood transfusion(s)	7	1	—	8
No transfusion	8	2	—	10
Died	20	7	1	28
Exchange transfusion	—	—	—	—
Blood transfusion(s)	4	—	—	4
No transfusion	6	3	—	9
Stillborn	10	4	1	15
Total	38	13	3	54

Pattern of the disease in 38 cases of Rh immunisation where the mother had a history of blood transfusion.

Hartmann and Brendemoen [29] studied the prognosis of post-transfusion infants born to Rh immunised patients who had received blood transfusions. 59 mothers had a total of 77 children of whom 5 at least were Rh negative. As the Rh groups of all the children had not been studied it is possible that a small number of the remaining 72 also were Rh negative. Out of them, 28 died either before or soon after birth and 44 recovered. Six of the former and 11 of the latter were transfused.

Freiesleben [26] and Hartmann and Brendemoen [29] have pointed out that in cases of immunisation by blood transfusion fairly definite conclusions on the prognosis of the child can be drawn from the mother's albumin titre. Hence it may be said that blood transfusion in the medical history of the mother does not indicate

a poor prognosis for the child unless the mother simultaneously reveals a high albumin titre.

A comparison of the number of children with a subclinical disease and of healthy children born to transfused patients with patients immunised by pregnancy alone would be of interest. The follow-up examination (page 29) showed that Rh immunised patients after blood transfusion had 24 Rh positive children. Five of them were healthy or had the disease in subclinical form. The present author feels that considerably more of these cases have been diagnosed in each group, now that routine examination during pregnancy has become generalised, than could be expected a few years ago.

By way of summary it may be concluded that the prognosis of the HDN children of transfused mothers is not essentially different from that of other HDN children, nor is there anything to favour the assumption that mothers immunised by blood transfusions have a larger number of healthy or subclinically diseased Rh positive children than those immunised otherwise.

5. Does the Foetus Act as an Immunisation Stimulus in Transfused Mothers?

Where the interval between the immunising blood transfusion and the subsequent pregnancy is short and where the antibody titre at the beginning of pregnancy is considerable it may be assumed that the foetus contracts the disease without acting as an immunisation stimulus at all. However, in the majority of the cases of transfusion immunisation this interval is so long that the antibodies at the beginning of the pregnancy are too weak to cause a disease of the severity generally found in the children of transfused mothers.

As a rule patients who have formed serologically demonstrable antibodies have been considered Rh immunised. If several years have elapsed from the last blood transfusion it is possible that the antibodies are so weak that even the most sensitive technique cannot demonstrate them. But in some cases, although she has received a blood transfusion or a blood injection, the patient forms no serologically demonstrable antibodies at all. In these

cases, however, the patient may have changed her mode of reaction in such a way that a serologically manifest immunisation sets in as a result of the next stimulus. As an example of such a case may be considered the person who is immunised by two blood injections. After the first she is not demonstrably immunised but has nevertheless changed her mode of reaction so that manifest immunisation is possible on the next injection. In these cases we are entitled to speak of a *sensibilisation* preceding immunisation, comparable to the sensibilisation of pregnancy immunisation discussed in the previous chapter. This opinion is supported by the follow-up examination material described above, in which a number of the Rh negative, non-immunised patients had evidently been sensibilised.

Hence, if a patient who has received blood transfusions, shows weak antibodies only or no demonstrable antibodies in early pregnancy, and the foetus nevertheless contracts clinical HDN, it is evident that the foetus has acted as a stimulus for immunisation. If this were not the case it would be difficult to explain why the child's prognosis deteriorates markedly after the first diseased child, as was evident e.g. from Chown's and the present author's materials.

The development of immunisation may also be observed by determining the mother's antibody titre at regular intervals during pregnancy. However, it must be pointed out that such determination, with the present technique, does not always give a result easy of interpretation. Although the amount of albumin antibodies and its fluctuations are often correlated with the severity of the child's disease there is no certainty of their being reliable indicators of the immunisation gaining in strength. Even if the amount of antibodies remains the same throughout pregnancy this does not preclude the foetus acting as an immunisation stimulus. Also, it is difficult to draw any conclusions from a rise or fall in titre during pregnancy. The present technique of determination yields very different results with different researchers, and doubtless often even with the same researcher. It is quite probable that the amount of circulating antibodies may vary for other reasons too during the pregnancy. An example of this is the so called »anamnestic reaction» [13,49] observable during a pregnancy in which the foetus is Rh negative and no antibody stimulus should exist. For natural reasons, the variations in the amount of antibodies have been observed primarily during pregnancy; hence it is not impossible for the so-called »anamnestic reaction» to be present in other conditions as well, either without a visible cause or as the result of a disease etc.

Hence, everything seems to support the assumption that an Rh positive foetus acts as an immunisation stimulus in the majority of cases, whenever immunisation or just sensibilisation has previously occurred as a result of blood transfusion or injection. The matter is of the greatest importance for the mechanism of pregnancy immunisation. *Pregnancy immunisation occurs in only a small fraction of the cases which in our opinion satisfy the conditions for immunisation. It is really remarkable that a large number of them are capable, even as a result of pregnancy, of forming antibodies producing disease in the foetus, once the first antigen stimulus has been given by blood transfusion.* It can therefore be considered certain that the rare incidence of pregnancy immunisation cannot result from foetal inability to act in the majority of cases as an immunisation stimulus. It can be held, rather, that the foetus, without the »releasing» effect of transfusion, is incapable of starting immunisation, of inducing in the mother the necessary change in mode of reaction, i.e. of acting as a sensibilising stimulus.

6. Intramuscular Blood Injections

The »releasing» effect of a blood injection on pregnancy immunisation is manifested with particular clarity in the cases where the injection is given intramuscularly. Although the materials of the majority of researchers doubtless contain such cases, few have been reported; Mollison[43], for instance, mentions just one[4]. If the patient has received only one intramuscular blood injection it must be considered probable that she has not formed any serologically demonstrable antibodies. If, subsequently, the first Rh positive child of such a patient is diseased the case can be considered a good example of the blood injection acting as a sensibilisation stimulus and the foetus as an immunisation stimulus. In what follows reports are given on some cases in which intramuscular blood injection seems to have caused a sensibilisation resulting in the child contracting the HDN.

Case 1. At the age of 8 the patient was given a »syringe-full» of blood intramuscularly as prophylaxis against poliomyelitis. The blood was taken from the patient's mother. First delivery 15 years later, healthy child. Second delivery after another 2 years — the child died at the age of 24 hours. Diag-

nosis on autopsy: erythroblastosis foetalis. Third delivery three years later. Weight of child 3,300 g. Blood count of the umbilical cord: Hb 14.2 g/100 cc. Er. 3,660,000. Leuc. 9,100. 39 nucleated cells per 200 leucocytes. Meulengracht 1: 10. Direct Coombs test strongly positive. Exchange transfusion was performed: the child showed a slight icterus; no further transfusions subsequently. At the age of two the child was normally developed. Results of blood group examination:

Mother	A cde/cde	Father	A CDe/cdE
1st child	A cdE/cde	Mother's mother	O CDe/cde
3rd child	A CDe/cde		

The mother was first examined serologically in the 6th month of her 3rd pregnancy. Albumin-agglutinating anti-D antibodies, titre 1: 64, were found in the serum. The titre remained unchanged during pregnancy.

As a child the patient had received Rh positive blood intramuscularly. Her first child was D negative and healthy. The second child suffered from severe erythroblastosis, a diagnosis made on autopsy. The following child had a reasonably mild HDN. The diagnosis was made serologically and clinically and the child recovered after an exchange transfusion.

Case 2. First delivery, healthy child. During her second pregnancy, in the 3rd month, the patient received 10 cc of her husband's blood intramuscularly. Gave birth to a macerated foetus a month before the calculated time. No autopsy but the placenta was found to be large and markedly lobed. Results of blood group examination:

Mother	A cde/cde
Father	O cDE/cde
1st child	O cde/cde

Serological examination was effected 9 months after the birth of the 2nd child. The mother's serum was found to contain albumin-agglutinating anti-D antibodies, titre 1: 16.

The patient, who had earlier given birth to an Rh negative child, was given 10 cc of her husband's blood intramuscularly in the 3rd month of her 2nd pregnancy. The foetus was born macerated. The large size of the placenta suggests HDN; no autopsy. Nine months later the mother was found to be Rh immunised, and the husband an Rh positive heterozygote.

Case 3. During her first pregnancy the patient was given two intramuscular injections of 10 cc of her husband's blood at an interval of two weeks in the 4th month. The child was vigorous on birth, became icteric on the

2nd day. No blood count performed. Recovered without blood transfusions. Results of blood group examination:

Mother	A cde/cde
Father	O CDe/cde
Child	O CDe/cde

Serological examination was effected 6 weeks before delivery, and the mother showed albumin-agglutinating anti-D antibodies titre 1: 16. Due to incomplete data the patient was delivered without our being informed, and hence a detailed serological and clinical examination was omitted.

During her first pregnancy the patient was given two intramuscular injections of her husband's Rh positive blood. The patient was found to be Rh immunised 6 weeks before delivery. The child evidently had mild HDN.

Case 4. At the age of 10 the patient was given a »syringe-full» of blood intramuscularly as prophylaxis against poliomyelitis. Gave birth, 10 years later, to a child who died during delivery. Diagnosis on autopsy was HDN. Foetal hydrops. The foetal serum was highly icteric. Results of blood group examination:

Mother	B cde/cde
Father	O CDe/cDE
Foetus	B cDE/cde, direct Coombs test strongly positive.

Serological examination was first effected in the 4th month of pregnancy. Weak anti-D antibodies were found. Titre in albumin 1: 1. Indirect Coombs test was positive. In the 6th month the strength of the antibodies was the same, in the 7th month albumin titre 1: 16, in the 9th month 1: 64 and 2 days before delivery 1: 256. Free albumin agglutinating anti-D antibodies were found in the foetal serum.

As a child the patient received 10 cc of blood intramuscularly. Her first child died during delivery of severe HDN; the diagnosis was confirmed both serologically and on autopsy.

CHAPTER III

ON FACTORS PROMOTING AND INHIBITING PREGNANCY IMMUNISATION

1. Survey of Literature

Numerous theories have been advanced to explain the fact that the majority of Rh negative mothers are not immunised by repeated pregnancies with an Rh positive foetus. To facilitate the discussion the theories may be divided into three categories:

1. Red cells containing Rh antigen enter the maternal organism in connection with every pregnancy; the rarity of the immunisation is a result of purely maternal factors.

2. Red cells containing Rh antigen enter the maternal organism in connection with some pathological occurrence only.

3. Variations are present in the strength of Rh antigen.

1. Levine [40] has estimated the surface area of the placental villi at 70 square feet and considers that in the majority of pregnancies therefore some of the foetal red cells must enter the maternal blood circulation. On the basis of his animal experiments Levine assumed that as little as 0.067 cc of packed red cells are capable of producing immunisation and that in every pregnancy an amount larger than this enters the maternal circulation from the villi. Wiener [76] was inclined to explain the low incidence of pregnancy immunisation by means of a hypothetical hereditary factor which supposedly determines the maternal immunisation ability. He denoted the gene with K, and presumed that homozygotes, who account for some 0.2 % of the population, produce antibodies easily, heterozygotes slightly less easily, while persons in whom the factor is completely absent — the majority — are totally incapable of immunisation. Pickles [49] has pointed out that such a factor does not explain the fact that half the Rh negative patients are capable of forming antibodies as a result of blood transfusion.

2. Potter [51], in a very interesting way, has discussed the possible role of various pathological occurrences during pregnancy and childbirth in Rh immunisation. It may be considered certain that abortions, either spontaneous or induced, may start immunisation. All materials on erythroblastosis contain cases in which immunisation began in the course of the pregnancy following an abortion, although the patient has had no previous pregnancies. The present author [47] has examined a case in which immunisation developed after three abortions, without any full-term pregnancy or blood transfusion. The pregnancies were twice interrupted artificially and once spontaneously, always before the 14th week of pregnancy.

However, in the majority of Rh immunisation cases the patient has had no abortion, and hence abortions suffice to explain a small number of immunisations only. Javert [32], in 8 cases of icterus gravis, found an intraplacental haematoma, and considered it possible that foetal red cells enter the maternal circulation in this way. Furthermore, Rh antigen may enter the maternal circulation when the placenta is separated in connection with childbirth or partly detached before delivery. Insofar as the separation damages the placental blood vessels and the decidua it facilitates the possible access of red cells into the maternal circulation, although this can hardly be shown. This may occur, e.g., in manual separation of the placenta, in connection with caesarean section or other irregularities of the second and third phases of parturition. Potter [51] in fact offers this explanation rather to show why the first child in cases of immunisation does not contract the disease and leaves open the question of the possible reasons for the rare incidence of immunisation.

3. Pickles [49], from results achieved in immunisation experiments with antigens other than D, considers it possible that such great variations may be present in the strength of D antigen too that they might suffice to explain the rare incidence of pregnancy immunisation.

Van Bolhuis [64] investigated whether extracts made of placenta and other organs reduced the titres of anti-Rh sera. He concluded that 10—15 % of the Rh positive have »Rhesus specific substances» in their organs. In cases where the child suffered from the haemolytic disease of the newborn as a result of the mother's

Rh immunisation he observed the inhibitory influence of the placenta in 31 out of 36 cases. According to him, the difference observable between the frequencies of blood transfusion and pregnancy immunisation could be explained by the fact that the placental Rh antigen induces the immunisation, and hence immunisation only occurs in patients with Rh specific substances in their organs.

Witebsky and Mohn [79] found water-soluble Rh substance, not only in the gastric juice but also in the amniotic fluid of a number of the cases studied, while in others it could not be shown. Witebsky [80] mentions that no Rh substance could be shown in cases where the child had erythroblastosis, although in approx. 85 % of the other patients examined this substance could be demonstrated by concentrating the amniotic fluid.

Smith [69] found that the anti-A and anti-B titres do not rise in the mother during and after pregnancy unless the child is a secretor. Mollison [43] advances the opinion that it must be called into question whether foetal red cells ever enter the maternal circulation. On the basis of investigations by Smith and by Mohn and Witebsky he considers it probable that Rh immunisation during pregnancy occurs through the action of soluble Rh substance, in which case this must be either weaker as an antigen than the Rh positive red cells or else it does not pass through the placenta in the normal way.

Before embarking on a detailed discussion of the reasons that might be responsible for the rare incidence of pregnancy immunisation compared with blood transfusion immunisation, the properties of pregnancy and transfusion immunisation described in Chapters I and II will be summarised, the summary serving as the basis for discussion.

2. Summary of Chapters I and II

1. An Rh positive foetus is a strong antigen stimulus in the cases where immunisation occurs.

2. In the majority of cases pregnancy immunisation occurs as a result of two Rh positive foetuses. In some cases several Rh positive foetuses are required to induce immunisation. The

probability of immunisation does not increase with the growing number of deliveries; indeed it seems to decrease.

3. Once the immunisation has taken place all subsequent Rh positive infants will be diseased.

4. In pregnancy immunisation the immunisation proper may be distinguished from sensibilisation, the preceding change in the mother's mode of reaction.

5. The majority of Rh negative persons are immunisable with blood injections and transfusions. If the patient has previously received a transfusion that has immunised or merely sensibilised her, her next consecutive Rh positive child as a rule will be affected with the haemolytic disease.

6. In these cases the foetus acts as the stimulus of immunisation, independent of whether or not the maternal serum contains Rh antibodies at the beginning of pregnancy.

3. Maternal Constitutional Factors

The effect of varying immunisability on the final result of immunisation manifests itself distinctly in experiments with blood injection immunisation. It is evident that a number of people are incapable of forming Rh antibodies, even after a good many injections. Hence it must be considered probable that a number of people are unable to form antibodies even through pregnancy. Although constitutional factors can be used to explain why some people are not immunised at all, the difference observed between the frequencies of pregnancy and blood transfusion immunisation cannot be attributed to these factors. The following points favour this conception:

— If constitutional factors are responsible for the rare incidence of pregnancy immunisation, the patients in whom immunisation occurs should be particularly good at forming antibodies. However, in some cases of pregnancy immunisation the mother proves to be a poor producer of antibodies, which is evident both from the low antibody titres and the good prognosis of the children.

— If a blood transfusion is given to a person who has not developed antibodies as a result of repeated pregnancies she

may, in connection with her next pregnancy, prove a very good producer of antibodies.

— Should all the foetuses act as stimuli of immunisation the immunisation probability will increase with the growing number of stimuli, even fairly steeply. The fact that no such increase is observable seems to indicate that in the majority of cases the foetus does not act as an immunisation stimulus at all.

— If red cells enter the maternal organism in all pregnancies, the foetus should give a considerably weaker stimulus than the blood transfusions. Where pregnancy immunisation occurs, however, the foetus constitutes an intense stimulus of immunisation, which is evident from the fact that the immunisation is sudden, and the mother's titre by the time the first child becomes diseased is in most cases relatively high and the prognosis of the child poor.

On the other hand, it must be pointed out that in the cases where immunisation has already taken place (either due to pregnancy or blood transfusion), all the subsequent foetuses act as immunisation stimuli. It would seem, therefore, that in the majority of cases the foetus is incapable of starting the immunisation, i.e. it cannot act as the sensibilising stimulus although, once the sensibilisation has occurred, it is capable of causing a serologically and clinically manifest immunisation.

4. Obstetrical factors: Immunisation is produced only by some pathological occurrence during pregnancy.

The possibility cannot be denied that an abrasion carried out in connection with abortion or some other traumata to which the placenta is subjected may in many cases be the ultimate cause of immunisation. The effect of such a pathological occurrence may be compared to a blood transfusion: it starts the immunisation or sensibilises the patient, in which case immunisation should follow from the next Rh positive foetus. In no case can it be expected that an investigation into the pregnancy during which the immunisation manifests itself will reveal such pathological occurrences to account for the immunisation. Naturally the investigation must deal with the preceding pregnancy, with an Rh positive foetus.

However, there are certain points that contradict the assumption that causes of this kind alone are responsible for the rare occurrence of pregnancy immunisation.:

— The probability that sensibilisation will occur in a known pregnancy can hardly depend on the consecutive order of the pregnancy. Hence the sensibilisation probability should remain unchanged, and with the immunisation manifesting itself from the next Rh positive foetus the probability of immunisation would remain the same no matter which the pregnancy in question. If the delaying effect on immunisation of heterozygous fathers is taken into consideration, the probability of immunisation should reveal a rising trend from the second pregnancy onwards.

— To date at least it cannot be considered probable that the various complications during pregnancy in cases of pregnancy immunisation are pronouncedly more frequent than in normal materials.

— The incidence of irregularities connected with pregnancy and delivery must be higher in hospital material than in any other materials. This should cause a difference in the incidence of immunisation of the different materials. As far as is known no such difference has been established, which of course may also be due to the fact that this particular aspect of the problem has not been studied.

5. Foetal Factors

If the rare occurrence of pregnancy immunisations is caused by foetal factors, there are two possibilities: the presence of some rare factor is conditional to the development of immunisation, or an ordinary factor inhibits immunisation (sensibilisation). Factors inhibiting immunisation are known to exist (A and B blood group factors). For this reason the following study will concentrate on the properties the factor inhibiting immunisation (sensibilisation) should possess for its effect to be compatible with the characteristics of immunisation mentioned at the beginning of this chapter.

The foetal factor inhibiting sensibilisation

As pointed out previously in several connections, Rh positive foetuses act as effective stimuli of immunisation provided sensi-

bilisation has occurred previously. The starting point, therefore, must be that the effect of »factors inhibiting immunisation» involves sensibilisation only. If sensibilisation occurs, whether as a result of pregnancy or blood transfusion, immunisation will follow from the next Rh positive foetus, independent of whether or not the inhibiting factor is present.

Should the absence of such a property, present in the majority of foetuses, be merely coincidental the situation would be the same in principle as when the obstetric factors mentioned under 4 above induce sensibilisation: it would lead to an increase of the probability of immunisation from the second pregnancy onwards. In addition, it is difficult to imagine that the effect of a factor governed by a mere coincidence could result in immunisation frequencies of roughly the same value in different materials.

In fact, the decrease in the probability of immunisation as the number of pregnancies increases — observed in the present material — supports the assumption that the factor inhibiting sensibilisation must be hereditary, and one that is determined by a single pair of genes. In the present author's opinion, this observed decrease in the probability cannot be explained in any other way.

Let us assume that the factor inhibiting sensibilisation is governed by a single pair of genes, inherited in compliance with Mendelian law; let us call this factor Xx.

The gene governing the sensibilisation-inhibiting factor can hardly be dominant (X). If it were, the majority of people would belong to genotype XX, and sensibilisation would follow with every foetus of genotype xx. As the inhibiting factor is common, both parents in the majority of marriages where sensibilisation occurs would be of genotype Xx, and the X factor would be absent from 25 % of the foetuses only. This would result in the sensibilisation not occurring, on an average, until the fourth pregnancy, which as is known is not the case.

Should the gene governing the inhibiting factor be recessive (x), sensibilisation could occur much earlier on the average. In that case the majority of people would belong to genotype xx, the predominant part of the balance to genotype Xx and a small part to type XX. A condition of sensibilisation would be that the foetus belongs either to genotype Xx or XX. As the x factor must be considered very common, the majority of Xx

children are born of marriages in which one of the partners represents genotype Xx and the other xx. 50 % of the children of such a marriage are of genotype Xx, which was the condition for sensibilisation. When the husband is homozygous to factor D sensibilisation will occur in 50 % of the cases in connection with the first pregnancy, in a quarter of them in connection with the second, in an eighth in connection with the third, etc., with immunisation manifesting itself one pregnancy later. A husband heterozygous to factor D will give the same result except that sensibilisation and the ensuing immunisation require an average of twice as many pregnancies before they develop.

a) *Effect of the sensibilisation-inhibiting factor on the frequency of immunisation*

Table 5, on p. 20, gives a calculation of how many mothers delivered in 1949 in Finland had a minimum of two Rh positive children. The result obtained was 411.5 computed per 1,000 Rh negative mothers. The following table gives a similar calculation of how many mothers delivered in Finland in 1949 had a minimum of two Rh positive children, of whom the penultimate or an earlier child belonged to genotype Xx while one of the parents belonged to genotype xx and the other to Xx.

TABLE 21

Parity	Per cent	Husband homozygote ‰	Husband heterozygote ‰	Total ‰
I	32.2	—	—	—
II	26.6	47.8	16.0	63.8
III	16.8	44.3	22.9	67.2
IV	9.2	29.0	19.0	48.0
V	5.5	18.6	15.1	33.7
VI	3.4	11.9	11.1	23.0
VII	2.3	8.1	8.6	16.7
VIII ≧	4.0	12.3	17.3	29.6
Total	*100.0*	*172.0*	*110.0*	*282.0*

Number of Rh negative mothers that have given birth to a minimum of two Rh positive children, of whom the penultimate or an earlier has belonged to genotype Xx (with one of the parents belonging to genotype xx and the other Xx). The relative sizes of the parous groups are taken from Table 5.

28.2 % only fulfil the condition made of immunisation.

The assumption above was that one of the parents belongs to genotype xx the other to type Xx. Obviously sensibilisation may also occur with other combinations: If one of the parents is XX and the other xx all the children will be of type Xx, in which case sensibilisation will result from the first positive foetus (cf. Table 5). If again both parents are of type Xx, 75 % of the children will be either XX or Xx. In order to form an idea of how large a percentage of the marriages in which sensibilisation can occur includes one xx and another Xx parent, some conception must be obtained of the gene frequency of x:

If we assume that e.g. 60 % of the Rh negative are capable in general of developing Rh antibodies, and that genotype XX is given a certain value, an idea can be obtained of the immunisation frequency and it can then be compared with known immunisation frequencies.

This estimate may be made as follows: Let us say $x = p$ and $X = q$. Assuming that genotype XX covers 0.2 % of the population, we obtain $q^2 = 0.002$ and $q = 0.047$. As $p + q = 1$, p is $= 0.953$ and $p^2 = 0.908$. As $p^2 + 2pq + q^2 = 1$, 2 pq will be $= 0.090$. The distribution of genotypes and marriage combinations will then be as follow:

xx	= 90.8 %	xx · xx	= 82.4 %
Xx	= 9.0 %	Xx · xx	= 16.3 %
XX	= 0.2 %	Others	= 1.3 %

With q^2 given the value 0.004 the distribution of genotypes and marriage combinations will be as follows:

xx	= 87.7 %	xx · xx	= 76.9 %
Xx	= 11.9 %	Xx · xx	= 20.9 %
XX	= 0.4 %	Others	= 2.2 %

In the marriage combination Xx · xx the immunisation frequency, according to Table 21, was 28.1 %. As 16 % of the population are Rh negative, 60 % of them were capable of forming antibodies, and the combination Xx · xx covered 16.3 %, the immunisation frequency will be

$$\frac{28.1 \cdot 16 \cdot 60 \cdot 16.3}{100 \cdot 100 \cdot 100 \cdot 100} = 0.45\ \%\ \text{or}\ 1:222.$$

With q^2 given the value 0.004, the corresponding figure is 0.50% or 1: 200.

The purpose of the calculation was to ascertain how many of the marriages in which sensibilisation is possible belong to the combination Xx · xx. In the former alternative this combination covered 16.3 % of all the combinations and the others 1.3 %, i.e. nearly 93 %, in the latter alternative 20.9 % and the others 2.2 %, or 90 %.

Although the effect of the sensibilisation-inhibiting factor on the immunisation frequency is completely decisive, no conclusions pro or contra the existence of the inhibitory factor can be drawn from the above discussion. The discussion, however, was an absolutely necessary preliminary to embarking on a study of the influence of the sensibilisation-inhibiting factor on the probability of immunisation.

b) *Effect of the sensibilisation-inhibiting factor on the probability of immunisation*

The *probability of immunisation* refers to the number of patients immunised for the first time as a result of pregnancy compared with the size of the corresponding parous group in the total material or population. By dividing the relative number of those immunised for the first time during a known pregnancy (compared with the total of the immunised) by the relative size of the corresponding parous group (compared with the total material or population) we obtain the *index of immunisation probability.*

The probability of immunisation is dependent of course on the frequency of the x gene, whereas the index of immunisation probability bears hardly any relation to it. Insofar as the variations caused by the x factor in this index in each parous group are the same as those of the corresponding indices in some clinical material this no doubt favours the existence of such a sensibilisation-inhibiting factor.

As Xx · xx evidently constitutes approx. 90 % of the marriage combinations in which sensibilisation may take place, this combination only is considered in the following.

The number of those immunised *for the first time* in each parous group in Finland in 1949 can be computed from Table 21. From

this we compute the percentage the figures obtained represent of the total of those immunised for the first time. Comparison of these figures with the relative values for the corresponding parous groups gives us the index of immunisation probability in each parous group (Table 22).

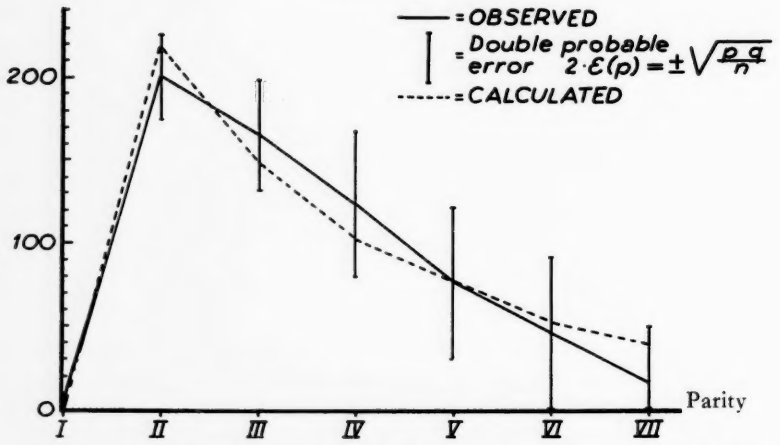
TABLE 22

Parity	Per cent	Husband homoz. ‰	Husband heteroz. ‰	Total ‰	Per cent	Index of probability
I	32.2	—	—	—	—	—
II	26.6	47.9	16.0	*63.9*	57.4	216
III	16.8	15.1	12.4	*27.5*	24.7	147
IV	9.2	4.1	6.6	*10.7*	9.6	104
V	5.5	1.2	3.4	*4.6*	4.1	75
VI	3.4	0.4	1.6	*2.0*	1.8	53
VII	2.3	0.1	0.9	*1.0*	0.9	39
VIII≷	4.0	0.1	1.6	*1.7*	1.5	
Total	*100.0*	*68.9*	*42.5*	*111.4*	*100.0*	

Number of *new* cases where an Rh negative mother has given birth to a minimum of two Rh positive children, of whom the penultimate belonged to genotype Xx when one of the parents belonged to genotype xx and the other to Xx. The index of probability has been obtained by comparing the relative sizes of these new cases with the relative sizes of the corresponding parous groups observed in Finland in 1949.

To illustrate the matter, the following diagram contains a comparison of the indices thus obtained with the indices computed from the present author's material (Table 3). Double probable error has been entered on both sides of the indices obtained from the present clinical material.

DIAGRAM II



Observed (Table 3) and calculated (Table 22) values of the index of immunisation probability in the different parous groups.

The similarity in the course of the curves is conspicuous. However, this is doubtless also due to coincidence, as there are many error factors which could not be taken into account in computing the immunisation probability indices from the author's own material:

— Whenever the material is collected from a population group of comparatively few multiparae and, accordingly, relatively more numerous secundiparae and tertiparae the immunisation probability index does not change in the way estimated above. The extreme in this respect (with the fewest multiparae) is observed in Helsinki. Even if the immunisation probability index is computed from the relative parous group sizes in Helsinki the index will fall, although not very steeply.

— It must be considered possible that the present material contains relatively too few cases in which the immunisation only started after the birth of several Rh positive children. It is quite probable that submission of a sample is not considered important in such cases, as there already are several healthy children in the family, or the physician in charge, because of »atypical medical history», never thinks of the possibility of immunisation.

— In the present material abortions have been included in the number of births preceding immunisation. But it has not been possible in calculating the size of the parous groups of the total

population to include the abortions, as their number is not known. The number of pregnancies among the population is greater than the statistics employed here would indicate. This naturally makes for a steeper fall in the indices of immunisation probability than that calculated above, compensating for the influence of the above two error factors.

c) *A and B blood group factors as examples of a foetal factor inhibiting sensibilisation*

As early as in 1943, Levine [39] found that the number of incompatible marriages among erythroblastotic families is considerably lower than might be expected. Many subsequent investigations have confirmed this. The following table shows the relative number of incompatible marriages in the materials of the various researchers:

TABLE 23

Pourcentages prévus de combinaisons matrimoniales:		Compatibilité du systeme ABO	Incompatibilité du systeme ABO
Pourcentages trouvés par:	Nombre de cas examinés	(66 %) %	(34 %) %
Bessis	48	89.5	10.5
Levine	215	75	25
Race	247	84	16
Wiener	96	71	29
Broman	33	81	19
Van Loghem et Spaander	240	87	13

The AB compatibility and incompatibility in families with haemolytic disease of the newborn due to Rh immunisation. (From van Loghem and Spaander [65]).

As groups A and B are relatively numerous in Finland the number of incompatible marriages is higher than in Central Europe. According to Streng [61] the distribution of the blood groups is as follows: O = 32.5, A = 41.9, B = 18.2, AB = 7.4, and p = 29.0, q = 13.8, and r = 57.2. In an unselected material, therefore, approx. 40.7 % represent incompatible marriages.

In the present author's material [47] of 195 erythroblastotic families in which the mother had received no blood transfusions, 39, or 20.0 % were incompatible.

It is therefore evident that the foetus's A and B properties

may inhibit Rh immunisation, provided the corresponding factor is absent from the mother. Many explanations have been advanced for the phenomenon [25,75,65,3]; the mechanism of the inhibitory influence need not be discussed in this connection, but we will examine the reasons why the inhibitory influence is observable only in approx. half the cases of paternal incompatibility.

If one of the children of incompatible parents contracts the haemolytic disease all the following children will also contract it, no matter whether they are incompatible or compatible. Neither is it probable, once immunisation has taken place, that an incompatible child will act as a weaker stimulus of immunisation than a compatible. *The inhibitory effect of A and B factors, therefore, doubtless affects sensibilisation only;* once it has occurred these factors are no longer capable of inhibiting the manifestation of immunisation, and hardly of moderating its strength. In the cases where sensibilisation has been induced by blood transfusion or blood injection, accordingly, no difference should be apparent between the immunisation frequency of incompatible and compatible marriages. The present author's material [47], which only covers 28 incompatible marriages in which the mother has received a blood transfusion and the next positive child has contracted the haemolytic disease, supports such a mechanism: incompatible and compatible marriages totalled 14 each, and hence no inhibitory effect was observable.

In many incompatible marriages the husband is heterozygous to the inhibitory factor. In such cases half the children are incompatible, the other half compatible. If a compatible foetus is Rh positive, there is no obstacle to sensibilisation. According to the above, the next child will be diseased, whether compatible or not. Schiff [82] and Wiener [68] have shown that, given known values for p, q and r, the distribution of mother-child combinations in the population can be computed. The number of incompatible children, computed according to Schiff [82], is as follows:

Group of Mother	Children's Groups		
	A	B	AB
O	pr	qr	
A		pqr	pq(p+r)
B	pqr		pq(q+r)

Inserting the values obtained in Finland for p, q and r in the table, we find that 24.8 % of the children only are incompatible. As 40.7 % of the marriages in Finland are incompatible, only three-fifths (60.9 %) of the children born of these marriages are incompatible.

The author's material of 39 erythroblastotic families [47] with incompatible fathers included only 7 families in which the first diseased child was not preceded by at least one compatible child. The inhibitory influence on sensibilisation, therefore, was observable in approx. five-sixths of the cases. Although the material, in spite of its limited range, supports the author's conception of the mechanism of the inhibitory influence, it indicates without doubt that the *A and B factors are incapable of inhibiting sensibilisation in some cases.* It is possible that these factors are not in themselves capable of inhibiting sensibilisation, and that the presence of some other factor is required, a factor whose incidence corresponds approximately to the frequency of the inhibitory effect observed. Taking into account the incidence of the *secretor* property (80—85 %) and its other characteristics it could be thought that the inhibitory influence of A and B factors is observable only if the foetus is simultaneously a secretor. This is supported by Smith's [60] observation that a serologically demonstrable rise in anti-A and anti-B titres is found in the mother during pregnancy only if the foetus, in addition to the A or B property, possesses the secretor property. The present author has not been in a position to study experimentally the occurrence of the secretor property in the incompatible children of immunised mothers. Although a study of the secretor properties or Lewis groups of the parents may provide indications of how the secretor property is capable of inhibiting sensibilisation, the investigation, if it is to provide a conclusive answer, must naturally focus on the healthy, Rh positive children preceding the first diseased child, who in addition are incompatible vis-à-vis the mother as regards their A or B groups.

A comparison of the characteristics of the hypothetical x factor and the foetal A and B blood group factors and their influence on Rh immunisation shows that:

1. Both of them are foetal hereditary factors governed by a single gene pair.

2. Both influence sensibilisation: once this has occurred for one reason or another, neither of them is capable of inhibiting the manifestation of the immunisation itself.

3. Both of them cause a similar delay in immunisation and decrease in immunisation probability. As was pointed out above, the father in incompatible marriages where Rh immunisation has taken place must be (mostly at least) heterozygous to the inhibitory A or B factor. In such a case the immunisation can be effected with 50 % by the first foetus, with 25 % by the second, with 12.5 % by the third, etc., and is manifested one pregnancy later. The situation, therefore, is completely similar to that assumed with the x factor, when one of the parents was of genotype xx and the other Xx.

6. Discussion

In the study of the mechanism of maternal immunisation due to the foetal Rh antigen the principal attention has usually been devoted to the pregnancy during which the immunisation manifests itself or to later pregnancies still when the immunisation has already occurred in the previous pregnancies. Similarly, observations on the frequency of immunisation include all the cases of immunisation, irrespective of whether the immunisation manifested itself for the first time during the pregnancy examined, or earlier. The present investigation approaches the mechanism of immunisation from a more or less new angle, devoting special attention to two conceptions: sensibilisation and the probability of immunisation. As they have been dealt with in various contexts in this work, a few of the reasons which led the author to introduce these new conceptions are reported on in the following.

In order to develop, pregnancy immunisation requires a minimum of two stimuli by an Rh positive foetus. The change induced in the maternal mode of reaction by the primary stimulus is termed sensibilisation in the present work, whereas the antibody formation induced by the next stimulus and demonstrable both serologically and clinically has been termed the immunisation proper. Many factors, dealt with separately in the foregoing,

favour the primary or sensibilising stimulus playing a more important part in the mechanism of immunisation than the subsequent stimuli:

— After the first diseased child, all the following foetuses act as stimuli of immunisation.

— If the primary stimulus is given by means of blood transfusion all the subsequent foetuses, in the majority of cases, will be effective stimuli.

The latter point shows clearly on what the attention must be focussed when embarking on a study of the reasons for the rare occurrence of pregnancy immunisation: there can hardly be any doubt that the rare occurrence of pregnancy immunisation must be due to the fact that the foetus is incapable of inducing sensibilisation in the mother, in the majority of cases, although once the latter has occurred all the foetuses in the majority of cases are capable of acting as immunisation stimuli.

The present work took the consideration of how the number of pregnancies affects the probability of immunisation as its starting point for the study of the factors affecting sensibilisation. Prior to that, however, it was necessary to define probability of immunisation and how it differs from the conception of immunisation frequency. A study of the various factors affecting the frequency of immunisation showed that frequency of immunisation cannot give any idea of how large a percentage of persons is capable of forming Rh antibodies through pregnancy. Above all it showed that the increase found in the frequency of immunisation with the number of pregnancies increasing does not justify any conclusions as to the variations in immunisation probability. In these circumstances it was explicable that in the present material immunisation probability declined from the second pregnancy onwards, an observation which, as far as is known, has not been reported before.

Basing an investigation of the factors that may be responsible for the rare occurrence of pregnancy immunisation compared with transfusion immunisation on this foundation it would seem that various features of the mechanism of immunisation are most readily explicable from hypothetical, sensibilisation-inhibiting or -promoting foetal factors, which in addition would be simply hereditary. On the other hand there is no doubt that

other factors also, expressly constitutional and obstetric factors, play a part in the etiology of immunisation.

No consideration has been given in the foregoing to the possible nature and mode of action of such factors as affect sensibilisation. Nor would it have been possible, as the author has made no experimental investigation into this aspect. However, it may be mentioned that van Bolhuis's[64] Rh substance may perhaps be suggested as a factor promoting sensibilisation, as it was demonstrable in the cases where the child was affected with HDN. The substance demonstrated by Witebsky[80] in the amniotic fluid and elsewhere may perhaps be assumed to inhibit sensibilisation; it could not as a rule be shown in the amniotic fluid of immunised patients. The foetal A and B blood group factors have been mentioned above as examples of sensibilisation-inhibiting factors, but their action involves a small number only of the Rh negative mothers. On the basis of analogy it could be assumed that some known or to date unknown blood group factors might inhibit sensibilisation in the same way.

In conclusion it may be mentioned that the author has no intention of claiming that the existence of the hypothetical foetal factors affecting sensibilisation has been proved by the present work. Indeed this was not its purpose. Besides conceptional clarification of the various factors affecting immunisation, this investigation merely endeavours to attract attention to such features of immunisation which, investigated in more detail theoretically and especially experimentally, may throw light on the many problems of pregnancy immunisation still awaiting solution.

Summary

After a short general introduction, the first and second chapters of the present paper deal with the properties of pregnancy immunisation, concentrating on two conceptions: sensibilisation and probability of immunisation.

Starting from the fact that immunisation requires a minimum of two antigen stimuli from Rh positive foetuses, the first of these is called the sensibilising, the second the immunising stimulus. In the majority of cases Rh positive foetuses do not in-

duce immunisation of the Rh negative mother. In the cases where the mother is immunised the diseased child is often preceded by a single, but frequently by two, three and even more, Rh positive healthy children. In his consideration of these points the author arrives at the following conception which, apart from observations reported by various researchers, is based on two sets of material collected by the author: one that covers 246 cases of pregnancy immunisation plus 38 cases in which the mother had received a blood transfusion prior to the birth of the diseased child, and another comprising 509 female patients of fertile age who had received one or more transfusions:

An immunisation proper, demonstrable both serologically and clinically, is always preceded by a change in the maternal mode of reaction induced by the former stimulus, sensibilisation. In the majority of cases the foetus is incapable of inducing sensibilisation of the mother. In the cases where sensibilisation occurs the immunisation manifests itself through the next Rh positive foetus. In these cases it is often the first foetus that causes sensibilisation, but in certain cases only the second, third or an even later foetus is capable of acting as the sensibilisation stimulus.

In contrast to pregnancy immunisation, a transfusion induces maternal sensibilisation in the majority of cases, manifest enough to be demonstrable serologically even. Once sensibilisation has occurred in this way all the subsequent Rh positive foetuses act as immunisation stimuli.

In immunisation cases the stimulus given by pregnancy must be considered a very good stimulus of immunisation (and sensibilisation). This is evident from the suddenness of the immunisation and from the relatively poor prognosis of even the first diseased child.

Probability of immunisation refers to the number of the immunised patients in whom the immunisation manifested itself *for the first time* during the pregnancy observed compared with the number of Rh negatives of the same parous group in the total material (or their proportionate number in the population). The frequency of immunisation, on the other hand, includes *all* the cases of immunisation; in a number of them the immunisation has manifested itself during the previous or still earlier pregnancies. The author concludes that the frequency of immunisation,

in these circumstances, depends on both the immunisation probability and the fertility of the immunised patients (i.e. on the probability of the immunised patient giving birth to yet another child).

On this basis it is considered evident that the frequency of immunisation, to start with at least, rises steeply as the number of pregnancies increases. By means of the indirect method of calculation worked out by the author it is then established that the *probability* of immunisation in the present material falls fairly steeply from the second pregancy onwards, particularly if the immunisation-delaying influence of heterozygous husbands is taken into consideration.

The third chapter deals with the reasons that, on the basis of the foregoing observations, may account for the rare occurrence of pregnancy immunisation compared with transfusion immunisation. Starting again from the conception that this difference must be due to the rare ability of pregnancy to induce maternal sensibilisation, the maternal constitutional factors and purely coincidental obstetric factors are considered from this standpoint. As the established difference is only partially explicable by this means the author concludes that the explanation is to be found primarily in foetal, sensibilisation-inhibiting factors. The observed drop in the probability of immunisation with the number of pregnancies increasing, in the author's opinion, can only be accounted for by the fact that the foetal factors in question must be simply hereditary.

The characteristics are discussed of such a hypothetic, sensibilisation-inhibiting factor, assuming that it is bound to a single pair of genes and inherited according to Mendelian law. Particular attention is devoted to the way in which such a property might affect immunisation probability and the frequency of immunisation, and the immunisation probability estimated by the method of calculation developed by the author is compared with the author's own clinical material.

In conclusion, the foetal A and B blood group factors are suggested as examples of factors inhibiting sensibilisation. The author advances the assumption that these factors, without the presence of the secretor property, are incapable of inhibiting sensibilisation, basing himself on his own limited material.

In a separate vein, the author deals with the various factors affecting the frequency of immunisation, endeavouring at the same time to estimate the range of their influence. Special attention is paid to the changes in the frequency of immunisation attributable to the variations in size of the parous groups; the changes are assessed by the method of calculation developed by the author. The finding arrived at is that investigations of the frequency of immunisation can never provide accurate information on the percentage of the Rh negative female patients capable of forming Rh antibodies through pregnancies alone, nor on the potential dissimilarities in this respect between different races or nations. The probability of immunisation in the various parous groups and its variations, on the other hand, might provide a considerably more accurate picture of these matters.

The relative number of homozygous husbands of the immunised patients is discussed as an indicator of the influence of the factors affecting the frequency of immunisation, and reference is made to certain points of importance in assessing the probable error in genotyping husbands of immunised patients.

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